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Dopamine as a Prolactin (PRL) Inhibitor

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Dopamine is a small and relatively simple molecule that fulfills diverse functions. Within the brain, it acts as a classical neurotransmitter whose attenuation or overactivity can result in disorders such as Parkinson's disease and schizophrenia. Major advances in the cloning and characterization of biosynthetic enzymes, transporters, and receptors have increased our knowledge regarding the metabolism, release, reuptake, and mechanism of action of dopamine. Dopamine reaches the pituitary via hypophysial portal blood from several hypothalamic nerve tracts that are regulated by PRL itself, estrogens, and several neuropeptides and neurotransmitters. Dopamine binds to type-2 dopamine receptors that are functionally linked to membrane channels and G proteins and suppresses the high intrinsic secretory activity of the pituitary lactotrophs. In addition to inhibiting PRL release by controlling calcium fluxes, dopamine activates several interacting intracellular signaling pathways and suppresses PRL gene expression and lactotroph proliferation. Thus, PRL homeostasis should be viewed in the context of a fine balance between the action of dopamine as an inhibitor and the many hypothalamic, systemic, and local factors acting as stimulators, none of which has yet emerged as a primary PRL releasing factor. The generation of transgenic animals with overexpressed or mutated genes expanded our understanding of dopamine-PRL interactions and the physiological consequences of their perturbations. PRL release in humans, which differs in many respects from that in laboratory animals, is affected by several drugs used in clinical practice. Hyperprolactinemia is a major neuroendocrine-related cause of reproductive disturbances in both men and women. The treatment of hyperprolactinemia has greatly benefited from the generation of progressively more effective and selective dopaminergic drugs. (*Endocrine Reviews* 22: 724–763, 2001)

Abbreviations: AF, activator function; BPA, bisphenol A; CCK, cholecystokinin; CNS, central nervous system; COMT, catechol-*O*-methyl transferase; CSF, cerebrospinal fluid; DAT, dopamine transporter; DBD, DNA binding domain; DBH, dopamine β-hydroxylase; DDC, L-aromatic amino acid decarboxylase; D_2L, D_2-type long isoform; DOPA, dihydroxyphenylalanine; DOPAC, dihydroxyphenylacetic acid; D_1R, D_1 receptor; D_2R, D_2 receptor; D_2S, D_2-type short isoform; ED, embryonic days; EGF, epidermal growth factor; eNOS, endothelial NO synthase; ERE, estrogen response element; GABA, γ-aminobutyric acid; GAT, GABA transporter; GRP, gastrin-related peptide; HBD, hormone binding domain; hGH, human GH; 5-HT, 5-hydroxytryptamine; JAK, Janus kinase; MAO, monoamine oxidase; MPP^+, 1-methyl-4-phenylpyridinium; NGF, nerve growth factor; NO, nitric oxide; NSF, *N*-ethylmaleimide-sensitive factor; OP, octylphenol; OVEX, ovariectomized; PHDA, periventricular-hypophysial dopaminergic; PRF, PRL releasing factor; PRL-R, PRL receptor; αSNAP, α soluble NSF attachment proteins; SCN, suprachiasmatic nucleus; SNARE, SNAP receptors; stat, signal transducer and activator of transduction; TERPs, truncated ER products; TH, tyrosine hydroxylase; THDA, tuberohypophysial dopaminergic; TIDA, tuberoinfundibular dopaminergic; TM, transmembrane; TMD, TM domain; VEGF, vascular endothelial growth factor; VMAT, vesicular monoamine transporter.

I. Introduction

DOPAMINE, NOREPINEPHRINE, AND epinephrine belong to a class of neurotransmitters known as catecholamines, which are structurally defined by a catechol ring and an amine side chain. Catecholamines and indolamines (*i.e.*, serotonin) are referred to as monoamines. Monoamines are small, water-soluble molecules that are the decarboxylated derivatives of amino acids. Production from their respective amino acids is catalyzed by several enzymes that act in sequence, the first of which serves as the rate-limiting step. Monoamines are stored at high concentrations in secretory granules. These granules provide protection against degradation by metabolic enzymes and enable a regulated release via exocytosis. Like other neurotransmitters, monoamines act very rapidly and their action can be terminated by both metabolic conversion to inactive compounds as well as by reuptake into the producing cell.

Dopamine is synthesized primarily in the central nervous system (CNS), but limited production also occurs in the adrenal medulla. Dopamine is also detectable in a few non-neuronal tissues, *e.g.*, the pancreas and the anterior pituitary.

Dysfunction of dopaminergic systems is associated with a number of diseases. For example, deficiency of dopamine in midbrain nigrostriatal neurons has long been recognized in the pathogenesis of Parkinson's disease, while overactivity of the limbic and cortical dopaminergic neurons has been implicated in schizophrenia and psychoses. These dopaminergic neurons are also affected by neurotoxins, psychostimulants, and drugs of abuse. In the neuroendocrine axis, dysfunction of hypothalamic dopamine or its pituitary receptors leads to hyperprolactinemia and reproductive disturbances. It is not surprising, therefore, that this relatively simple molecule has been at the center of interest of basic scientists and clinicians alike for many years.

Within the brain, catecholamines function as classical neurotransmitters, *i.e.*, they communicate between neurons and act within the anatomically confined space of the synapse. However, by virtue of their presence in the circulation and action on distant target organs, catecholamines from the adrenal medulla were among the first compounds classified as hormones in the early 1900s. Not until the 1970s, however, did the role of dopamine as an inhibitor of the pituitary lactotrophs become recognized. Since then, dopamine has been clearly established as the primary regulator of PRL gene expression and release. On the other hand, among the many factors capable of stimulating PRL, none has emerged as a leading candidate for a PRL releasing factor (PRF). Therefore, PRL homeostasis should be viewed in the context of a fine balance between the action of dopamine as an inhibitor and the many hypothalamic, systemic, and local factors acting as stimulators.

In 1985, we published a review in this journal entitled "Dopamine: A Prolactin Inhibiting Hormone" (1). The present update covers pertinent information that has been gathered since the publication of this report. During the last 15 yr, this field has witnessed unparalleled progress, including the cloning of dopamine and PRL receptors (PRL-Rs), the characterization of the dopamine transporter, the recognition of the role that estrogen and its receptors play in PRL homeostasis, and the generation of transgenic animals deficient in all these genes. In terms of therapeutic applications, dopaminergic agonists have become the mainstay treatment for suppressing PRL in hyperprolactinemic patients and for shrinking prolactinomas.

The review is organized in five chapters. The first chapter covers advances in the understanding of dopamine synthesis, storage, release, reuptake, and receptor binding. The second and third chapters focus on the hypothalamo-pituitary dopaminergic systems, their regulation by various factors, and the mechanisms by which dopamine affects the lactotrophs. Lessons learned from transgenic animals with altered genes that are relevant to PRL regulation constitute the fourth chapter. Finally, the profile of PRL release in humans, clinical aspects of dopaminergic drugs, and the pathophysiology of hyperprolactinemia are presented in the fifth chapter. Since 1985, over 4000 articles have been published on various aspects of dopamine-PRL interactions. By necessity, this review is selective rather than inclusive. Consequently, the reader is referred whenever possible to other reviews for more in-depth coverage of the different topics.

II. Characteristics of Dopaminergic Neurons

A. Synthesis and metabolism

Dopamine biosynthesis begins with the amino acid tyrosine (see Fig. 1). The majority of circulating tyrosine originates from dietary sources, but small amounts are derived from hydroxylation of phenylalanine by the liver enzyme phenylalanine hydroxylase (for review, see Ref. 2). Tyrosine enters neurons by an energy-dependent uptake process and

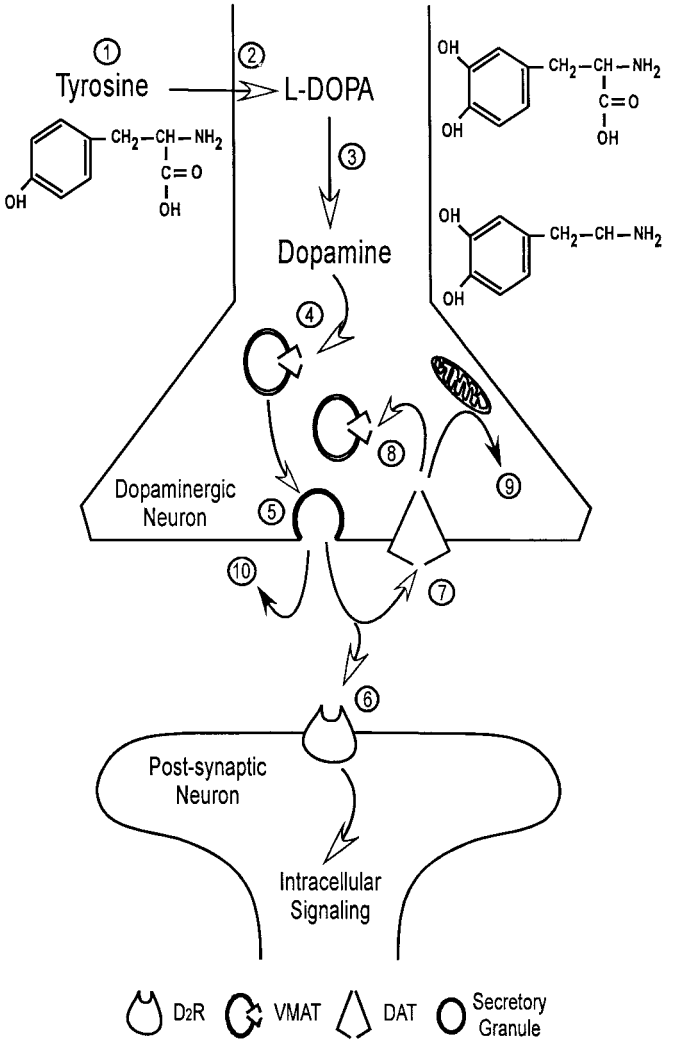

FIG. 1. Diagram of dopamine biosynthesis, release, and metabolism. 1) Tyrosine is taken into the neuron by a sodium-dependent mechanism; 2) conversion of tyrosine to L-DOPA by TH is the rate-limiting step in the biosynthetic pathway; 3) L-DOPA is converted to dopamine by DDC; 4) dopamine is translocated into secretory vesicles for storage, protection, and secretion; 5) fusion of secretory vesicles with the plasma membrane results in dopamine release into the synaptic cleft or the extracellular space (as is the case with the TIDA neurons); 6) dopamine binds to its membrane receptors and initiates multiple effects in target cells; 7) unbound dopamine is taken up by the DAT, located in the plasma membrane of the presynaptic neuron; 8) both newly synthesized dopamine and that taken up into the cell are translocated into secretory vesicles by the VMAT; 9) MAO, located in the outer mitochondrial membrane, converts dopamine to a deaminated metabolite; 10) COMT converts dopamine or its deaminated metabolite to biologically inactive products.

is converted to dopamine by two enzymes that act in sequence, tyrosine hydroxylase (TH) and L-aromatic amino acid decarboxylase, also called dihydroxyphenylalanine (DOPA) decarboxylase (DDC). Neurons that contain active dopamine β-hydroxylase (DBH) convert dopamine to norepinephrine, and those that also contain phenylethanolamine *N*-methyl transferase convert norepinephrine to epinephrine. The latter are classified as noradrenergic and adrenergic neurons, respectively, and their distribution in the brain differs considerably from that of the dopaminergic neurons. Regardless of the catecholamine being produced, TH is the rate-limiting step in their biosynthetic pathway.

TH is a mixed function oxidase that uses tyrosine and molecular oxygen as substrates (for review, see Ref. 3). The cofactor tetrahydrobiopterin (BH4) donates the hydrogen atom needed for hydroxylation of tyrosine to DOPA. Because pterin also serves as a cofactor for other monoxygenases as well as nitric oxide synthase, its availability is a determining factor in the control of TH activity (4). Tyrosine itself is not a limiting factor because TH is virtually saturated at the normal plasma concentrations of tyrosine. This explains why tyrosine administration is not an effective treatment for alleviating dopamine deficiency in Parkinson's disease or for suppressing PRL release in hyperprolactinemic patients. Amino acid analogs of tyrosine, *e.g.*, α-methyl-*p*-tyrosine, inhibit TH by competing with the tyrosine substrate and are useful for assessing dopamine turnover rate. This is based on the concept that the exponential rate of decline in tissue dopamine after TH inhibition is proportional to neuronal activity. The early reports of changes in dopamine turnover rates in the hypothalamus were instrumental in establishing a reciprocal relationship between dopamine and PRL release under many conditions (reviewed in Ref. 1).

The TH gene is localized to chromosome 11p in humans and encodes a single form of TH that can be alternatively spliced (5). Targeted disruption of the TH gene results in perinatal lethality, which can be rescued by L-DOPA administration (6). The mature enzyme is a soluble cytosolic protein composed of four subunits of approximately 60 kDa each (reviewed in Ref. 7). Each monomer is comprised of an inhibitory regulatory domain at the N terminus and a catalytic domain at the C terminus. The regulatory domain contains four phosphorylation sites located within the first 40 amino acids: Ser^{8}, Ser^{19}, Ser^{31}, and Ser^{40}. The catalytic domain contains the pterin binding region and a putative leucine zipper at the C terminus that participates in intersubunit binding.

TH activity is the most critical factor that controls dopamine synthesis, and considerable efforts have been devoted to understanding activation/inactivation of this enzyme. TH activity is regulated by two mechanisms: short-term activation and long-term induction (for review, see Ref. 8). Activation (seconds to minutes) occurs in response to increased nerve impulses or pharmacological agents and involves removal of feedback inhibition by dopamine, allosteric regulation by polyanions, and phosphorylation. All phosphorylation sites, but especially Ser^{40}, are important for TH activation (9). Phosphorylation at specific sites is accomplished by several Ser/Thr kinases, *e.g.*, PKA, PKC, ERK1/2, and calcium calmodulin-dependent protein kinase II, and results in conformational changes that alter enzyme affinity either to the pterin cofactor or to dopamine acting as an inhibitor (4). Because TH phosphorylation can be reversed by phosphatases, the activated state of TH at any given time reflects a dynamic balance between these antagonizing forces. As discussed in *Section II.B*, TH appears to be constitutively activated within the hypothalamo-pituitary unit, in sharp contrast with its normally quiescent state in both the striatum and adrenal medulla.

Long-term induction of TH involves transcriptional regulation, alternative RNA splicing, RNA stabilization, and translational regulation (for review, see Ref. 8). Nucleotide sequences up to 9 kb upstream from the transcriptional start site are necessary for developmental and tissue-specific control of TH expression. The promoter region contains several positive and negative transcriptional elements that are not conserved across species and differ among tissues (10). Changes in TH mRNA levels occur in response to alterations in physiological conditions, *e.g.*, cold exposure and chronic stress, and are often mediated by glucocorticoids (11). Three major second messenger systems, cAMP, diacylglycerol, and calcium, which use a variety of effector molecules, have been implicated in this response. A combination of enzyme activation and long-term induction maintains dopamine synthesis by diverse and seemingly redundant tissue-specific mechanisms. These overlapping actions guarantee uninterrupted supply of the neurotransmitter and permit rapid neuronal responsiveness to many physiological stimuli.

As shown schematically in Fig. 1, DDC is the second and terminal enzyme in dopamine biosynthesis (reviewed in Ref. 12). The enzyme uses pyridoxal phosphate as a cofactor and can convert both DOPA to dopamine and 5-hydroxytryptophan to serotonin [5-hydroxytryptamine (5-HT)]. Although a single gene codes for the enzyme, there are several isoforms that may be responsible for preferred decarboxylation of either dopamine or serotonin. The mature enzyme is a dimer made of 50-kDa subunits and is regulated by *de novo* synthesis rather than by changes in its activity. Under basal conditions, enzyme activity is so high that L-DOPA is virtually undetectable.

Unlike dopamine, DOPA can cross the blood brain barrier, and this property has been exploited in the treatment of Parkinson's disease, especially during the early stages when a sufficient number of midbrain dopaminergic neurons are still functional. To prevent rapid decarboxylation, DOPA has to be administered together with peripheral DDC inhibitors such as carbidopa or benserazide (13). Other DDC inhibitors, *e.g.*, NSD 1015, have been widely used in laboratory animals for measuring DOPA accumulation as an index of dopamine biosynthesis (14). This approach is based on the findings that DOPA levels without drug application are virtually undetectable. Measurement of DOPA accumulation is well suited for evaluating dopaminergic neuronal activity in the median eminence and posterior pituitary, which contain only minimal levels of norepinephrine.

Catabolism is one of the effective mechanisms for dopamine inactivation (reviewed in Ref. 15). This involves multiple pathways that include oxidative deamination by monoamine oxidase (MAO), *O*-methylation by catechol-*O*-methyl transferase (COMT), and conjugation by sulfotransferases or glucoronidases. The preferred metabolic pathway at a given

site depends on the compartmentalization of the metabolic enzymes. For example, MAO is located in the external membrane of the mitochondria and acts intracellularly, whereas COMT is associated with the external cell membrane and acts only extracellularly.

MAO exists as two isoenzymes, A and B, with an apparent molecular mass of 60–63 kDa each. The two MAO genes, each comprised of 15 exons, are located on the X-chromosome and appear to have been derived from the same ancestral gene (reviewed in Ref. 16). They differ in substrate specificity as well as selectivity for inhibitors. MAO-A is more highly expressed in catecholaminergic neurons, whereas MAO-B is more abundant in serotonergic and histaminergic neurons and in glial cells (17). Enzyme inactivation in humans or its deletion in transgenic mice are compatible with life but result in neurochemical and behavioral abnormalities (16). Deamination of dopamine by MAO produces dihydroxyphenylacetic acid (DOPAC). Determination of the ratio of DOPAC/dopamine concentrations serves as a good method for estimating rapid changes in neuronal activity, with a major advantage being that it does not require drug pretreatment. *O*-Methylation by COMT is primarily responsible for inactivation of circulating catecholamines. Consecutive conversion of dopamine by MAO and COMT yields homovanillic acid.

B. Storage and exocytosis

Because most endocrinologists are more familiar with peptide/protein hormones than with neurotransmitters, it is appropriate to compare the different characteristics of storage and exocytosis of these two classes of compounds. Dopamine is stored in secretory vesicles at a 100- to 1000-fold higher concentration than neuropeptides. This is attributed to several distinct features of monoaminergic neurons. First, unlike neuropeptides whose synthesis occurs within the endoplasmic reticulum and Golgi apparatus, dopamine biosynthesis can take place within the terminals themselves. Second, synthesis that occurs in a close proximity to the site of release permits a much faster turnover rate than the slow axoplasmic transport that brings proteins from cell bodies to the nerve terminals. Third, a unique reuptake process replenishes most of the released dopamine back into the secretory vesicles and maintains high intragranular concentration, whereas a released neuropeptide cannot be restocked.

After synthesis, dopamine is stored in synaptic vesicles at extremely high concentrations, 0.5–0.6 M, which is near its limit of solubility. Dopamine is translocated from the cytoplasm into the vesicles by the vesicular monoamine transporter (VMAT), shown schematically in Fig. 1 and discussed in detail in *Section III.C*. The function of the vesicles is 4-fold: 1) to protect dopamine from enzymatic degradation by MAO, 2) to minimize constitutive secretion by diffusion from the cells, 3) to facilitate regulated release, and 4) to enable rapid replenishment of depleted stores. The life cycle of the vesicles includes: 1) targeting to the active zone of the presynaptic membrane, 2) docking, 3) fusion, 4) release of the vesicular content, 5) retrieval by endocytosis, and 6) refilling with the neurotransmitter. Selected aspects of these events are discussed below. For a comprehensive coverage, please refer to several outstanding reviews (18–21).

Monoamines are stored primarily in small translucent ("clear") vesicles (50–100 nm in diameter) but are also present in large dense core vesicles (up to 500 nm in diameter), often cosequestered with neuropeptides (22). The relationship between large and small vesicles, their predominance in catecholaminergic *vs.* neuroendocrine cells, their membrane composition, and their precise role in quantal neurotransmitter release are not clear. Also, most information on synaptic vesicles has been obtained from chromaffin cells, which contain primarily norepinephrine and epinephrine and differ from dopaminergic neurons by the presence of intravesicular DBH, chromogranins, and other constituents (23). It remains to be determined whether the content of the vesicles and the process of exocytosis are identical in dopaminergic and noradrenergic neurons.

Storage vesicles are formed in the neuronal perikarya and are transported to the terminals by slow axoplasmic flow. Although early studies suggested formation of vesicles from the outer membrane of the terminals by pinocytosis, it was later realized that this represented retrieval by endocytosis of previously fused vesicles. In fact, to maintain adequate transmitter storage and permit a sustained response to stimuli, endocytosis must occur at a rate that parallels exocytosis. The synaptic vesicle is a highly specialized structure whose membrane is composed of a lipid bilayer with embedded integral proteins that participate in vesicular trafficking, docking, and fusion. The vesicle membrane also contains an H^+-ATPase, which maintains the proton gradient that energizes VMAT and preserves an acidic intravesicular environment. Each vesicle is filled with several thousand molecules of dopamine as well as other soluble constituents (24).

Much information has been gathered in recent years on the docking mechanism (reviewed in Refs. 18 and 25–27). It involves a family of proteins termed α soluble *N*-ethylmaleimide-sensitive factor (NSF) attachment proteins (αSNAP) receptors (SNARE) complexes: v-SNAREs, designating vesicular-associated proteins, and t-SNAREs, designating target (plasma membrane) cognate complexes. At least seven to eight proteins are essential for docking: vesicular synaptobrevin and synaptotagmin; SNAP-25 and syntaxin, which are located in both the vesicles and plasma membrane; and two soluble proteins, NSF and SNAP, which catalyze the disassembly of the SNAP-25-syntaxin-synaptobrevin complex during docking and fusion (28). SNAP-25, in association with syntaxin, binds to and modulates voltage-gated calcium channels, thus bringing the vesicle into close proximity with a source of calcium. Both N-type and P/Q-type calcium channels have been implicated in neuronal exocytosis, with synaptotagmin I acting as a low-affinity calcium sensor (29).

The critical role of calcium in exocytosis, termed the "stimulus-secretion coupling" hypothesis, has been long recognized. Calcium is central to all aspects of exocytosis, including rapid fusion and unloading of the vesicles as well as recruitment and translocation of loaded vesicles. Resting levels of cytoplasmic calcium within the neuron are approximately 0.1 μM and can rise to 5–10 μM upon arrival of action potentials (19). Calcium influx occurs through voltage-gated calcium channels and leads to fusion of the synaptic vesicles

with the plasma membrane and release of their content to the extracellular space. This is a much faster process than the relatively slow release of peptide or protein hormones from endocrine cells.

In most neurons, dopamine is released into the synaptic cleft and binds to postsynaptic receptors. In contrast, the dopaminergic neurons of the hypothalamo-pituitary unit (with the exception of the dopaminergic neurons innervating the intermediate lobe of the pituitary) lack true synaptic contacts and are classified as secretory neurons (30). In this case, dopamine diffuses away from the terminals through the perivascular space and is transported by portal blood to distal pituitary target cells. The rate of dopamine release from secretory neurons appears to be slower than that from classical neurons. It has been argued that the speed of neurotransmitter release is reciprocally related to the distance of its site of action, but the mechanism responsible for this feature is unclear (19).

Calcium influx in chromaffin cells induces an initial fast release, termed the "exocytotic burst," which occurs in milliseconds and is followed by a slower and sustained release phase that lasts several seconds (27). It is assumed that only a small fraction of docked vesicles can instantaneously release their cargo in response to calcium influx. These vesicles comprise the "fusion-ready" pool that undergoes a very rapid ATP-dependent fusion. The slower release phase is carried out by docked vesicles that exist in a different biochemical state and require priming to promote fusion. These vesicles constitute a precursor pool that replenishes the rapid release pool. Priming is ATP dependent, involves the SNARE proteins, and is associated with production of phosphoinositides and protein phosphorylation. An even slower pool is composed of vesicles that are anchored to the cytoskeleton via actin-binding synapsins but are not docked to the membrane (25). When synapsins become phosphorylated in response to an influx of calcium, the vesicles detach from the cytoskeletal elements and can translocate to the active zone of the presynaptic membrane. However, vesicular translocation is too slow to account for the immediate calcium-dependent exocytosis.

Norepinephrine is released from chromaffin granules together with ATP and chromogranins but it is unclear whether this also occurs in dopaminergic neurons (23). The dynamics of release has been studied by electrophysiological approaches capable of resolving single exocytotic events. Such techniques can detect small changes in membrane capacity, reflecting an increase in plasma membrane surface due to vesicular fusion, and can also measure the oxidation/reduction potential of minute amounts of the released transmitter (31). Two pathways have been proposed to explain formation of fusion pores that connect the vesicle lumen with the extracellular space. One is termed the "kiss-and-run pathway," in which a pore is formed to allow partial or full emptying of the vesicle content. The other is termed the "complete fusion pathway," in which the pore dilates and the vesicle membrane collapses into the plasma membrane (18, 32). At least two membrane proteins, synaptophysin and synaptoporin, have been associated with pore formation. Questions that remain to be resolved include the three-dimensional structure of the putative pores, the precise mechanism of vesicular retrieval, and the dynamic forces that drive intracellular trafficking of the internalized vesicles.

C. Transporters

Reuptake is the process by which the released transmitter is brought back into presynaptic nerve terminals or is internalized by surrounding glial cells. It is unique to monoamines and amino acid neurotransmitters and is the main mechanism by which the action of the released transmitter is rapidly terminated (see Fig. 1). As an added benefit for monoamines, reuptake permits recycling of the same molecules while saving in energy costs of their biosynthesis (33). In contrast, the action of the released neuropeptide is terminated either by diffusion or by proteolysis.

Reuptake of dopamine is mediated by two classes of transporters: dopamine transporter (DAT), which transports dopamine from the extracellular to the intracellular space, and VMAT, which reloads dopamine into the vesicles (reviewed in Ref. 34). The two transporters differ in structure, cellular localization, substrate specificity, antagonist selectivity, and energy requirements (for a schematic presentation of the two transporters, see Fig. 2). Because dopamine in the hypothalamo-pituitary axis is not released directly into synapses, it has been argued that reuptake is not physiologically important in these neurons (35). As discussed in more detail in *Section III.B*, compelling evidence now indicates that a dopamine reuptake mechanism, though not robust, is an essential component in the overall regulation of PRL homeostasis.

The search for membrane transporters began after observing rapid uptake of labeled catecholamines into brain slices and synaptosomes. This reuptake was Na^+ and Cl^- dependent and inhibited by cocaine and amphetamine (reviewed in Refs. 36–38). The importance of reuptake was underscored by dramatic physiological and behavioral effects of several drugs of abuse that interfere with this process in both humans and laboratory animals. These observations lead to the notion that the presynaptic membrane must contain distinct molecules that act as symporters, *i.e.*, they have the capacity for concentrating the transmitter by a concurrent movement of Na^+ down its electrochemical gradient (38). The process of uptake has an apparent stoichiometry of $2Na^+$:$1Cl^-$:1dopamine, suggesting an electrogenic process (39).

The γ-aminobutyric acid (GABA) transporter (GAT) was the first neuronal transporter isolated by classical protein purification methods, followed by cloning of the norepinephrine transporter, NET (reviewed in Refs. 36, 37, and 40). After screening cDNA libraries from the rat midbrain with oligonucleotide probes complementary to conserved regions of these transporters, DAT was cloned by three groups in 1991 (41–43). Many neuronal transporters have since been cloned and are now grouped into a large family characterized by multiple transmembrane domains (TMDs) and sodium dependence. DAT belongs to a subfamily that includes transporters for GABA, norepinephrine, serotonin, glycine, and proline and is distinguished by 12 TMDs and dependence on both Na^+ and Cl^-. Another subfamily is chloride independent, has 6–9 TMDs, and includes transporters for the excitatory amino acids glutamate and aspartate (40).

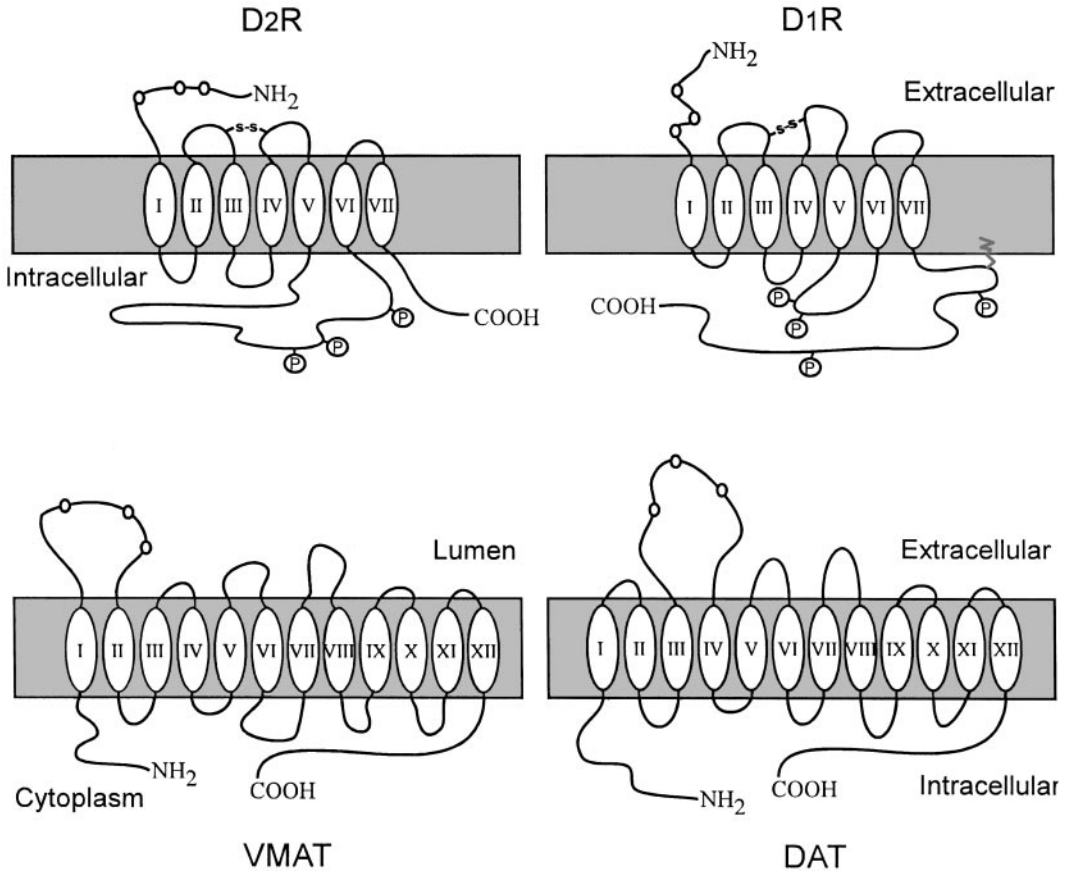

FIG. 2. Comparison of the structures of dopamine receptors (*upper panel*) and transporters (*lower panel*). Receptors: Both D_1Rs and D_2Rs belong to the superfamily of 7 TMDs, G protein-coupled receptors. D_2Rs have a larger third intracellular loop, whereas D_1Rs have a longer cytoplasmic carboxyl terminus. The locations of N-glycosylation (*open circles*), putative phosphorylation (*circles with embedded P*) sites, the s-s bridge in the extracellular face, and site of palmitoylation in the cytoplasmic face are also shown. Transporters: Both DAT and VMAT have 12 TMDs but otherwise have little primary sequence homology. Unlike the receptors, both the N- and C-termini of the transporters are located at the cytoplasmic side of the membrane. DAT has a large, N-glycosylated extracellular loop between TMDs 3 and 4, whereas the largest intraluminal loop of VMAT is located between TMDs 1 and 2. *Open circles* designate glycosylation sites.

The DAT gene in humans is mapped to chromosome 5p (44). It spans 64 kb and is made of 15 exons, with the coding region beginning in exon 2 and extending partially into exon 15. There is a close correspondence between the exons and the putative TMDs, with no evidence for multiple start or polyadenylation sites or alternative splicing. The proximal 5′-flanking sequences lack canonical TATA or CAAT boxes and contain only a few known response elements for transcription factors (45). More distal sequences have multiple binding sites for nurr1, an orphan nuclear receptor transcription factor that is critical for the development of midbrain dopaminergic neurons (46), although it is not expressed in the hypothalamic dopaminergic neurons (47). A combination of positive and silencing elements within the promoter region accounts for the selective cellular localization of DAT within the brain. Although a number of potential transcription factor response elements (Erg-1, E-box, AP-2) have been identified in the proximal DAT promoter, their precise role in the regulation of DAT gene expression has not been well defined (45).

DAT encodes a 69-kDa protein of 620 residues with both the N and C termini located intracellularly (reviewed in Ref. 48; see Fig. 2). The protein lacks a consensus signal sequence and has 3–4 potential N-linked glycosylation sites in the second large extracellular loop. The nature and extent of glycosylation are tissue specific and may be involved in transporter targeting, stability, or ligand binding. Residues within TMD 1–3 influence binding affinity for dopamine and cocaine, whereas those in TMD 11–12 affect the affinity for the 1-methyl-4-phenylpyridinium ion (MPP^+) neurotoxin (49, 50). As is typical for all neuronal transporters, DAT has lower affinity and reduced ligand specificity than the dopamine receptor. Several potential sites can be phosphorylated by PKC and may determine the rate of uptake or serve as a signal for transporter internalization (51).

As revealed by combined *in situ* hybridization and immunocytochemistry, DAT has restricted localization within the brain and is not expressed outside the CNS (34). The transporter colocalizes with TH, and because it is limited to dopaminergic neurons, it serves as a unique marker for these neurons. The highest expression of DAT is in the substantia nigra, followed by the ventral tegmental area. DAT in these neurons is detected in perikarya, dendrites, and axonal processes. A significant presence of DAT is also seen in mesolimbic and mesocortical dopaminergic pathways (52), whereas the hypothalamic dopaminergic neurons exhibit

moderate and restricted expression of DAT. Unexpectedly, electron microscopy reveals that the DAT protein is found primarily in the extrasynaptic area rather than in the active zone of the synapse (53). This suggests that DAT may play a role in limiting diffusion of dopamine after being released. An unresolved issue is the mechanism by which dopamine (and other monoamines) is taken up by glia, because DAT is undetectable in these cells.

DAT is targeted by psychostimulants such as cocaine and amphetamine. By binding to the transporter and preventing dopamine reuptake, these drugs cause a prolonged increase in extracellular dopamine, resulting in augmentation of its effects. Because DAT is an excellent marker for functional extrahypothalamic dopaminergic neurons, *in vivo* imaging of cocaine analogs is used to evaluate the state of dopaminergic neurons in patients with Parkinson's disease and other neurological disorders (54). The generation of transgenic mice with DAT inactivation added significant information on the physiological role of this transporter. These mice are hyperactive, do not respond to cocaine or amphetamine, are resistant to the neurotoxic effects of MPP^+, and their dopamine receptor expression is down-regulated (55). The state of PRL and other hormones of the hypothalamo-pituitary axis in such animals is covered in detail in *Section V.A*.

Two VMAT isoforms, VMAT1 and VMAT2, were identified by expression cloning. They arise from distinct but related genes that encode proteins of ≈520 residues (reviewed in Refs. 33 and 56). Although they also have 12 putative TMDs, there is no sequence homology with the membrane transporters. The vesicular transporters are characterized by a large hydrophilic N-glycosylated intraluminal loop between TMDs 1 and 2, with both C and N termini located on the cytoplasmic side of the vesicular membrane (see Fig. 2). VMAT1 is present in developing neurons, peripheral tissues, and in some endocrine cells. VMAT2 is expressed in all major monoaminergic neurons throughout the brain, with a broad specificity for monoamine transport rated as serotonin> dopamine>norepinephrine>epinephrine>histamine (34). The weak substrate specificity indicates that uptake and storage of a secreted transmitter by the appropriate neuron is determined by the cell-selective plasma membrane transporter rather than by the vesicular transporter. Unlike DAT-deficient mice, which survive into adulthood, deletion of VMAT2 results in early postnatal mortality, underscoring the obligatory role of vesicular storage of monoamines for survival (57–59).

VMAT recharges the vesicles with the neurotransmitter by using an electrochemical gradient generated by vacuolar ATP-dependent H^+ pump (V-ATPase). This pump maintains an intravesicular acidic environment (pH of 5.5 in chromaffin granules), which is necessary for uptake of the transmitter against its concentration gradient (60). Hence, VMAT differs from DAT and other membrane transporters that are driven by a Na^+ gradient. Uptake into the vesicle involves extrusion of two protons for each transmitter molecule that is taken in. The low pH also facilitates packaging/storage of the vesicular content, but it is unclear whether vesicular alkalinization plays a role in exocytosis. In contrast to cocaine and amphetamine, which inhibit dopamine uptake by binding to DAT, the antihypertensive drugs reserpine and tetrabenazine inhibit uptake by interacting with VMAT (61).

D. Receptors

Studies in the late 1970s revealed binding of labeled dopamine to two receptors that were distinguished by pharmacological, physiological, and biochemical criteria and became known as D_1 and D_2 (62). It was then recognized that the D_1 receptor (D_1R) was coupled to G_s proteins and increased intracellular cAMP levels, whereas the D_2 receptor (D_2R) interacted with G_i proteins and inhibited cAMP accumulation. The D_2R was cloned 10 yr later by adopting a cloning strategy based on sequence homology to known G protein-coupled receptors (63). Cloning of the D_1R by several groups followed (reviewed in Ref. 64). Expression of the D_1R and D_2R in host cells confirmed their specificity for the various pharmacological agents and contrasting effects on adenylyl cyclase activity. Since then, three additional dopamine receptors were cloned and characterized. At present, there are 5 distinct receptors that are grouped into two subfamilies: the D1-like family, which includes D_1 and D_5, and the D2-like family, which includes D_2, D_3, and D_4. Because the regulation of PRL by dopamine is mediated by the D_2R, its properties will be emphasized (for structural comparison of D_1R and D_2R, see Fig. 2).

The dopamine receptors are members of the superfamily of G protein-coupled receptors. They are made of single polypeptide chains that range in size from 387 to 475 residues. The receptors have 7 transmembrane (TM)-spanning helices that form a ring-like hydrophobic pocket surrounded by 3 intracellular and 3 extracellular loops. The extracellular amino terminus in all dopamine receptors contains a similar number of residues but has a variable number of N-glycosylation sites (reviewed in Refs. 64 and 65). The cytoplasmic carboxyl terminus is much longer in D_1-like receptors and is the site of receptor anchorage to the plasma membrane via palmitoylation of a conserved cysteine residue (66). The third intracellular loop (between TMs 5 and 6) is significantly larger in the D_2 subfamily, as is the case for most receptors that interact with G_i proteins (Fig. 2). Several phosphorylation sites on the third intracellular loop and the cytoplasmic tail participate in receptor desensitization, although the physiological importance of desensitization is best established for adrenergic receptors (67). Two cysteine residues on the second and third extracellular loops form a disulfide bond that stabilizes receptor conformation. A combination of site-directed mutagenesis and protein modeling suggests that conserved amino acids in TM 2 (aspartate), TM 3 (aspartate), TM 5 (two serines), and TM 6 (phenylalanine) define a narrow pocket for agonist binding (64, 68).

The five dopamine receptors have different chromosomal localization, *i.e.*, 5q, 11q, 3q, 11p, and 4p for D_1, D_2, D_3, D_4, and D_5, respectively (69). It has been proposed that most of the genes encoding G protein-coupled receptors originated from a primordial gene, probably one of the opsin genes (70). The genomic organization of the dopamine receptors is consistent with their divergence from two gene subfamilies that differ in the presence or absence of introns. Like most G protein-coupled receptors, the D_1-like receptor genes have no introns, whereas the D_2, D_3, and D_4 receptors have 6, 5, and 3 introns, respectively (71). The presence of introns permits generation of receptor variants by alternative splicing.

D_2R has two functional variants, a short isoform, and a long isoform having a 29-residue insertion in the third cytoplasmic loop (72). Expression, regulation, and signaling of these variants within the anterior pituitary are covered in *Section IV.A*. Splice variants of other dopamine receptors may generate nonfunctional proteins.

In general, the D_1 and D_2 receptors are expressed at higher levels and have more selective agonists and antagonists than the D_3, D_4, and D_5 receptors (reviewed in Ref. 64). The five receptors have distinct, though often overlapping, localization within the brain and are expressed in a tissue-specific manner in the periphery. D_2R mRNA is highly expressed in the substantia nigra, ventral tegmental area, and hippocampus, whereas the amygdala contains primarily D_1 with only little D_2 mRNA. Both receptors are expressed at high levels in the caudate putamen, nucleus accumbens, and olfactory tubercle (73). The hypothalamus has moderate levels of both D_1 and D_2 mRNAs and low levels of D_4 and D_5. D_2R mRNA is expressed at high levels in both the anterior and intermediate lobes of the pituitary and at lower levels in the adrenal and retina. The D_3 receptor mRNA is not detected in peripheral tissues, whereas a low expression of D_1 and D_4 in the kidney and D_5 in the heart has been reported (74).

The association of dopamine receptors with many neurological disorders has led to the development of many agonists and antagonists (reviewed in Ref. 75). While the availability of stereoselective drugs was instrumental in the initial characterization of the receptors, their cloning subsequently helped in the discovery of more selective drugs. Dopamine receptor antagonists are known as neuroleptics and are widely prescribed for the treatment of schizophrenia and other psychoses (76). Because many neuroleptics elicit Parkinsonian side effects, *i.e.*, rigidity and akinesia, it led to the development of newer drugs, known as atypical neuroleptics, with little or no adverse effects on motor functions (77). The most common antagonists of D_2R are (+)butaclamol, chloropromazine, haloperidol, spiperone, sulpiride, and raclopride whereas apomorphine, bromocriptine, pergolide, and cabergolide are potent D_2R agonists (reviewed in Ref. 64). As discussed in *Section VI.B*, the latter compounds are very effective in the treatment of prolactinomas. Neither of the above-mentioned drugs is absolutely specific for any dopamine receptor subtype, and their selectivity is based on differences in binding affinity and dissociation constants to the various receptors. Thus far, there are no drugs that discriminate between the two D_2R variants.

Signal transduction by the dopamine receptors is an active area of research (reviewed in Refs. 78 and 79). As mentioned before, early studies using brain and pituitary tissues established that activation of D_1-type receptors increased adenylyl cyclase activity, whereas activation of the D_2-type receptors resulted in its inhibition. Because of receptor heterogeneity in brain neurons and lack of truly selective agonists and antagonists, transfection of non-neuronal cells with the various receptors has provided the bulk of information on receptor signaling. The major caveat is that many transfected host cells do not express the G proteins or downstream effectors that are physiologically relevant to receptors on neurons, often leading to conflicting results.

The ability of dopamine receptors to couple to appropriate G proteins is at the heart of their action. It is now recognized that a given receptor can be associated with more than one G protein, thus increasing its diversity of action. The D_1-like and D_2-like receptors are primarily associated with the $G_s\alpha$ and $G_i\alpha$ subunits, respectively. However, the G_0 and G_q proteins, which are associated with ion channels and phosphoinositide metabolism, are also involved (80). Many of the actions of dopamine receptors are extremely fast, involving rapid changes in ion fluxes across the cell membrane. In some target cells, including the pituitary lactotrophs, receptor activation also leads to changes in gene expression and hormone secretion as well as alterations in cell growth and differentiation. The signal transduction pathways of the pituitary D_2R and their multiple effects on the lactotrophs are covered in *Sections IV.A* and *IV.B*.

In contrast to the aforementioned receptors that are localized post synaptically, dopamine autoreceptors are found on most parts of the neuron, *i.e.*, soma, dendrites, and terminals (81, 82). Autoreceptors are divided into three subcategories that are classified by their ability to modulate dopamine synthesis, release, or neuronal firing rates. After being released from the neuron, dopamine can interact with autoreceptors and inhibits further release of the neurotransmitter. The elevated intracellular dopamine also suppresses TH activity by binding to its pterin cofactor and decreasing the rate of synthesis. Some prefrontal neurons, as well as the hypothalamic dopaminergic neurons, lack synthesis-modulating autoreceptors (83). Although both D_2 and D_3 receptors have been proposed to function as autoreceptors, the issue remains controversial. It is also unclear whether distinct receptor proteins modulate each of these functions or the same receptor protein is coupled to each function through distinct transduction mechanisms.

III. The Hypothalamo-Pituitary Dopaminergic Systems

A. Anatomy and ontogeny

The brain contains several well-defined dopaminergic systems. The most extensively studied are the nigrostriatal, mesolimbic, and mesocortical neuronal systems that originate in the midbrain and project to the striatum, limbic system, and cortex, respectively. These are involved in the control of locomotion, emotion, and cognition and have no direct role in the regulation of pituitary function. Within the hypothalamus, dopamine perikarya are located in several sites that are classified by the alphanumeric system of Dahlstrom and Fuxe (84). These include the posterior hypothalamus (A11), arcuate nucleus (A12), zona incerta (A13), periventricular nucleus (A14), and lateral and ventral hypothalamus (A15). Neurons arising from the A11 locus send axons to the spinal cord and constitute a diencephalospinal system whose function is not well understood. The incertohypothalamic neurons from the A13 group project diffusely to different areas of the hypothalamus and participate in the control of GnRH release. They also send some projections to more remote areas such as the amygdala. Although not well characterized, the neurons originating from A11 and A13 regions constitute the majority of the hypothalamic dopaminergic neurons (for

review, see Ref. 14). Only neurons originating from the A12 and A14 groups are relevant to our discussion because they are directly involved in the control of PRL secretion.

Based on anatomical and functional studies, two dopaminergic systems that regulate PRL were initially identified: the tuberoinfundibular dopaminergic (TIDA) and tuberohypophysial dopaminergic (THDA). Further refinements in neuronal tracing techniques (85) revealed that most of the THDA neurons projecting to the neurointermediate lobe actually originate from the A14 cells in the periventricular nucleus and therefore were termed the periventricular-hypophysial dopaminergic (PHDA) system (86). Because of their lower abundance and heterogeneous distribution, the hypothalamic dopaminergic neurons are not as well characterized as their nigrostriatal counterparts.

The TIDA neurons provide the major dopaminergic input to the anterior pituitary (see Fig. 3). Most of their perikarya are located in the dorsomedial part of the arcuate nucleus, with a smaller population arising from the periventricular nucleus. Their relatively short axons terminate in the external zone of the median eminence near the primary capillary loops of the hypophysial portal vessels (reviewed in Ref. 14). The median eminence contains a negligible number of cell bodies and a dearth of classical synapses. Instead, the TIDA system represents neurosecretory neurons whose product is released into perivascular spaces surrounding the capillary loops and is carried by the portal blood to the anterior pituitary. Notably, some TH-positive perikarya in the ventrolateral portion of the arcuate nucleus that also project to the median eminence do not express DDC (87) or DAT (88) and are believed to release DOPA rather than dopamine. Because DOPA has not been detected in hypophysial portal blood, the DOPA that may be released from these neurons must be decarboxylated before reaching the pituitary. Whereas the arcuate nucleus receives multiple afferent connections from

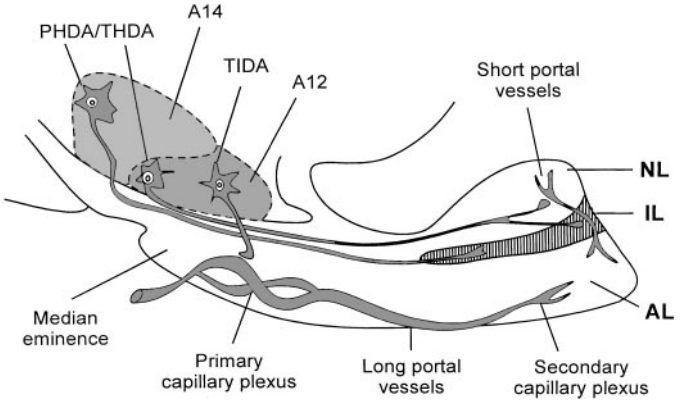

FIG. 3. Diagram of the hypothalamic dopaminergic systems that regulate PRL. The TIDA neurons originate in the A12 region of the dorsomedial arcuate nucleus. They have short axons that terminate in the external zone of the median eminence near the primary capillary plexus of the portal vessels. The released dopamine is carried out by the long portal vessels to the anterior lobe (AL) of the pituitary. The THDA/PHDA neurons have perikarya primarily in the A14 region of the periventricular nucleus and send projections to the neural (NL) and intermediate (IL) lobes of the pituitary. The short portal vessels connect between the NL and AL but bypass the IL which is avascular. [Derived from M. E. Freeman *et al.*: *Physiol Rev* 80:1523: 1631, 2000 (105)].

VIPergic, opioidergic, serotonergic, and NPYergic neurons among others, their precise connections to TH-positive neurons needs further clarification.

As depicted in Fig. 3, THDA/PHDA neurons project through the internal layer of the median eminence, course along the pituitary stalk, and terminate in the neural and intermediate lobes of the pituitary (85). Whereas the terminals in the neural lobe are neurosecretory, many of the terminals in the intermediate lobe form synaptic-like contacts with melanotrophs (30) and suppress their proliferation as well as inhibit β-endorphin and α-melanocyte-stimulating hormone release (89, 90). Notably, synaptic contacts between neurons and non-neuronal cells are uncommon. The presence of synapses as well as expression of electrical activity by the melanotrophs are more reminiscent of adrenal chromaffin cells than most endocrine cells. Although the exact cellular origin of the melanotrophs is unclear, by analogy to chromaffin cells, they may arise from neuroectodermal progenitors that lost their axons and migrated to an ectopic site.

The anatomy of dopaminergic neurons in the human hypothalamus has not been well characterized (reviewed in Ref. 14). Undoubtedly, dopamine is important for the regulation of PRL release in humans because drugs that interfere with its release or action affect circulating PRL levels to the same extent as they do in experimental animals. However, there is no clear demarcation of the A11–A14 dopaminergic groups in humans, and most TH-positive, DBH-negative neurons (designating dopaminergic rather than noradrenergic neurons) reside in the magnocellular neurons (91). However, it is clear that the hypothalamic dopaminergic neurons in humans have distinct properties from those in the nigrostriatal region, and their normal function is preserved in Parkinson's disease. Indeed, Parkinson's patients do not have elevated plasma PRL levels, and their basal PRL release is suppressed by dopaminergic agonists (92). Another point of departure from rodents is the absence of a distinct intermediate lobe in adult humans (as opposed to its discrete presence in fetuses) and no comparable PHDA system. Although melanotrophs are sparsely distributed throughout the human pituitary, they are neither innervated by dopaminergic neurons nor respond to dopamine agonists and antagonists (93).

Embryonic development of the hypothalamic dopaminergic neurons in the rat progresses along four chronological stages: 1) generation from neuroepithelial precursors, 2) expression of biosynthetic enzymes, production of dopamine, and establishment of mechanisms for its reuptake and release, 3) development of efferent connections, and 4) formation of afferent innervation and synaptogenesis (for review, see Ref. 94). Stage 1 neurons are first detected in the zona incerta and periventricular zone on embryonic days (ED) 12–13, followed by those in the arcuate nucleus on ED 14–15. On ED 17–18, TH-immunoreactive neurons (95) and TH mRNA (96) are detectable throughout the hypothalamus, with TH-positive axons appearing in the median eminence 2 d later. Expression of TH, however, does not imply terminal neuronal maturity. In fact, the high levels of DOPA in the fetal hypothalamus (97) and in conditioned media from cultured fetal hypothalamic neurons (98) suggest low activity of DDC and diminished ability for dopamine biosynthesis.

Stage 2 neurons expressing both TH and DDC and capable of release and reuptake of dopamine first become apparent on ED 15–16 (99). Sexual dimorphism of arcuate neurons is already evident at this time, with number of TH-positive neurons higher in males but their size and content higher in females (99). The gender difference in dopamine content is maintained during culture of fetal diencephalic neurons, which respond to chronic exposure to gonadal steroids by decreased rate of DOPA synthesis in cultures from both sexes (100).

The development of the THDA/PHDA system is delayed. TH-positive fibers are first seen in the neural lobe on ED 20 and in the intermediate lobe 3–4 d after birth (95, 101). Intermediate lobe-derived factors such as brain-derived neurotropic factor and neurotropin-3 may be involved in directing outgrowth of the dopaminergic neurons from the hypothalamus toward the pituitary (102, 103). The density of TH immunoreactivity in the intermediate lobe increases during the 1st wk of life, with dopamine reaching peak concentrations by the end of the 2nd wk and decreasing thereafter (104). The increased density of dopaminergic innervation in the intermediate lobe during early postnatal life correlates with the ontogeny of dopamine binding sites and coincides with a marked reduction in the number of melanotrophs (104), reflecting the inhibitory action of dopamine on melanotroph proliferation.

B. Physiology and pharmacology

Of the three dopaminergic systems, the TIDA neurons play the predominant role in the control of PRL release. Substantial evidence, based on the measurement of dopamine in portal blood and determination of its concentration and turnover rates in both the arcuate nucleus and median eminence, has established that the activity of the TIDA neurons is altered under many physiological conditions known to affect PRL release (reviewed in Refs. 1 and 105). However, the early notion that the TIDA neuronal activity must be negatively correlated with PRL release is too simplistic because the expected reciprocal relationship between dopamine and PRL is often masked by the action of other factors, both positive and negative, that control PRL release.

The lactotroph is unique among endocrine cells in having a high basal secretory activity. Tonic inhibition by dopamine, which maintains low circulating PRL levels, requires a continuous high input of dopamine. The high output, in turn, depends upon a sustainable high rate of synthesis. To enable rapid PRL surges, the dopaminergic input to the lactotrophs must be concomitantly decreased. This process is accomplished by a unique mechanism governing the regulation of hypothalamic TH activity. TH in most tissues exists in a quiescent, nonphosphorylated state. In response to stimuli, the enzyme is rapidly phosphorylated, resulting in increased hydroxylation of tyrosine to DOPA and its instant conversion to dopamine that is immediately available for release (9). One notable exception is hypothalamic TH. In keeping with the constant demand for high dopamine output, hypothalamic TH is constitutively active, as judged by its lower Michaelis-Menten constant (K_m) for the pterin cofactor than striatal TH (106). In response to estrogen, hypothalamic TH is transiently inactivated, presumably by dephosphorylation because this can be reversed by inhibitors of protein phosphatases such as okadaic acid (107). This is supported by the report on rapid decline in TH activity in hypothalamic slices within 1 h of E2 treatment (108). The absence of dopamine autoreceptors on the TIDA neurons may assist in maintaining high dopamine output by reducing, or eliminating, the negative feedback by dopamine on TH activity.

As discussed before, reuptake plays a fundamental role in neuronal function by conserving the released neurotransmitter and terminating its synaptic action. Because the hypothalamic neurons release dopamine into portal blood rather than into synapses, early reports suggested that that the hypothalamic dopaminergic neurons lack a functional reuptake mechanism (35, 109). This was refuted by a later study demonstrating dopamine reuptake by incubated stalk median eminence and posterior pituitary, with reuptake inhibitors such as nomifensin and diclofensin increasing media dopamine levels after neuronal depolarization (110). The molecular basis for this reuptake process was later confirmed by the detection of DAT mRNA in the dorsomedial arcuate nucleus (34) and the demonstration of immunoreactive DAT in the median eminence, pituitary stalk, and intermediate and neuronal lobes (111).

The physiological relevance of this reuptake was supported by the acute suppression of serum PRL levels in ovariectomized (OVEX) rats treated with competitive DAT inhibitors such as cocaine or mazindol (111). Moreover, DAT-knockout mice, presumably because of increased dopamine outflow, have a marked reduction in pituitary PRL content and do not lactate (112). Since the ratio of DOPAC/dopamine in the median eminence is less than half that in the striatum (113), most of the released dopamine must be carried away by the portal blood and lesser amounts are taken up by the terminals and converted to DOPAC.

Although males and females have the same density of TIDA nerve terminals, there are marked sexual differences in their activity and responsiveness to physiological and pharmacological stimuli. Basal TIDA neuronal activity is higher in females, is decreased by ovariectomy, and is restored by estrogen (for review, see Refs. 105 and 113). An opposite trend is seen in males, whereby TIDA activity increases by orchidectomy and decreases by T. The lower basal activity of these neurons in males may be due to tonic inhibition by endogenous opioids (114). The TIDA neurons in females are more sensitive to stress and to feedback stimulation by PRL but less sensitive to bombesin and κ-opioid antagonists (115, 116).

The TIDA neurons also exhibit an endogenous daily rhythm of activity (for review, see Ref. 105). This is controlled by the suprachiasmatic nucleus (SCN), which coordinates photoperiodicity in the neuroendocrine axis. A proestrous-like mid-afternoon PRL surge can be induced daily in OVEX rats by estrogen, implicating some form of coupling to an intrinsic diurnal rhythm. TIDA neuronal activity in the estrogen-treated OVEX rats is high in the morning, decreases before the PRL surge, but remains suppressed throughout late afternoon (117, 118). Although these changes correlate well with the initiation of the surge, they do not correspond to its termination, suggesting involvement of factors other

than dopamine. Progesterone also participates in the control of the TIDA rhythm by advancing the afternoon decline in TIDA activity (119). Such rhythmic activity does not occur in males and is abolished in females by lesions of the SCN (113). These data suggest that activation/inactivation of the TIDA neurons is driven by an endogenous rhythm that is independent of the reproductive state, but its amplitude and timing are modulated by ovarian steroids. Several factors, including opioid peptides, bombesin, and acetylcholine (113), whose actions may be mediated by nitric oxide (120), have been implicated in the control of the endogenous dopaminergic rhythm. The relative importance of all these factors as well as the hierarchy of their action remain to be defined.

The role of the THDA/PHDA neurons in PRL regulation has been controversial. Early studies failed to show a good correlation between THDA neuronal activity and PRL release, suggesting that they do not contribute to the control of PRL secretion (116). Other investigators, however, found that the concentrations of dopamine and DOPAC in the intermediate lobe exhibit a daily rhythm with a significant decline that coincides with the initiation of the proestrous PRL surge (121). They also found alterations in the DOPAC/dopamine ratio in both the intermediate and neural lobes in response to ovarian steroids (122) and PRL (123), indicating that all three populations of the dopaminergic neurons are involved with PRL homeostasis.

Unlike the long portal vessels that connect the median eminence to the anterior pituitary and can be cannulated, the short portal vessels linking the neural and anterior lobes are inaccessible for sampling. Therefore, the relative contribution of the THDA/PHDA neurons to the total dopamine reaching the anterior pituitary cannot be effectively determined and requires indirect approaches. Surgical removal of the posterior pituitary (posterior pituitary lobectomy or LOBEX) provided the first evidence that dopamine from the THDA/PHDA neurons suppresses PRL release. LOBEX in either male or female rats caused an increase in serum PRL levels, but not LH or GH, which was reversed by intracarotid injections of dopamine (124, 125). Another indirect method is compression of the pituitary stalk, which disrupts the neural input to the neurointermediate lobe but does not impede the blood supply to the pituitary (126). Within 1 wk of denervation, circulating PRL and α-MSH levels increased 3- to 4-fold, whereas those of LH remained unchanged. The mode of transport of dopamine from the avascular intermediate lobe to the anterior lobe remains enigmatic. One possibility is that it diffuses into the vicinity of lactotrophs that line the pituitary cleft. Another possibility is that intermediate lobe dopamine indirectly regulates PRL by suppressing the release of a local PRL releasing/regulating factor (reviewed in Ref. 127).

The pharmacology of the hypothalamic dopaminergic neurons has been the subject of several studies. These revealed that the responsiveness of the TIDA neurons to dopaminergic agents differs in several respects from their striatal counterparts. Because the TIDA neurons lack autoreceptors, they are unresponsive to acute administration of nonselective dopamine agonists, such as apomorphine, which do not discriminate between D_1-like and D_2-like receptors (128). Such drugs, therefore, act indirectly by altering the secretion of PRL, which in turns affects the TIDA neurons via a short loop feedback mechanism.

Classical antipsychotic drugs with D_2R antagonistic properties, such as haloperidol, have no direct effect on the TIDA neurons but induce their activation within several hours, secondary to the rise in circulating PRL levels (129). On the other hand, atypical neuroleptics such as clozapine acutely increase TIDA neuronal activity, possibly by activating D_1Rs. Acute administration of D_1 agonists (*e.g.*, SKF 38393) inhibits, whereas D_2 agonists (*e.g.*, quinpirole) stimulate, the TIDA neurons. The opposing effects of stimulatory D_2Rs and inhibitory D_1Rs likely account for the lack of net effect of mixed D_1R/D_2R agonists on the TIDA neurons (128). In males, some of the D_2R-mediated activation of the TIDA neurons occurs via disinhibition of afferent dynorphinergic neurons that provide tonic inhibition over the TIDA neurons (130).

C. Regulation by PRL

In the absence of target gland hormones to provide feedback control over the lactotrophs, PRL regulates its own release by acting on the hypothalamic dopaminergic systems. This type of interaction, termed "short loop feedback," is mostly responsible for the maintenance of PRL homeostasis. Many studies have established that an increase in either endogenous or exogenous PRL results in higher activity of the TIDA neurons, whereas a decrease in circulating PRL levels, resulting from hypophysectomy, immunoneutralization, or dopamine agonists, lowers their activity (reviewed in Refs. 1, 105, and 131). The TIDA neurons respond to both acute and chronic changes in PRL with few exceptions. The latter include pregnancy, lactation, and prolactinomas, when the dopaminergic neurons become refractory to the elevated PRL levels, thereby upholding physiological or pathological hyperprolactinemia.

The existence of a short loop feedback arrangement between PRL and dopamine raises several questions: Are the effects of PRL direct or indirect, and if indirect, what are the mediators? How does PRL gain access to the TIDA neurons? What are the nature and cellular distribution of hypothalamic PRL-Rs, and how are they regulated? Are both the TIDA and THDA/PHDA systems regulated by PRL? And finally, which functions of the dopaminergic neurons are affected by PRL? Presently, there are only partial answers to these questions.

The issue of direct *vs.* indirect effects of PRL on the TIDA neurons is difficult to resolve, given the scarcity of suitable *in vitro* systems. A direct effect is suggested by the increase in TH activity in cultured fetal hypothalamic neurons incubated with PRL (98). Although most of the TH-expressing neurons were immunopositive for the PRL-R, they constituted less than 10% of the total cell population in these cultures, and the receptors were also expressed by 20–25% of other neurons. In another study, dopamine concentration in TIDA neurons increased within 1 h after injecting ovine PRL to OVEX rats, indicating very rapid activation (123); a second delayed increase in dopamine turnover was seen in both the intermediate and neural lobes. Rapid activation by PRL of immediate early genes such as Fos-related antigens

(132) and nerve growth factor (NGF)1-A (133) in TH-positive neurons in the arcuate nucleus also demonstrates PRL autofeedback. Neither study, however, conclusively established a direct effect of PRL, and mediation by substances such as neurotensin, NPY, and opioids cannot be ruled out.

Circulating proteins are excluded from the brain proper except for the circumventricular organs, *e.g.*, the median eminence and neural lobe, which are outside the blood brain barrier. Given the location of the arcuate nucleus within the medial basal hypothalamus, it raises the question of how PRL reaches the TIDA neurons. PRL, which is detectable in many hypothalamic and extrahypothalamic sites (134, 135), can be derived from two sources: transport from the circulation, and local synthesis. Transport to the cerebrospinal fluid (CSF) occurs by receptor-mediated PRL uptake at the choroid plexus (136), which has the highest density of PRL-R in the brain (137, 138). The mechanism by which this receptor acts as a transporter is unknown. Upon gaining access to the CSF, PRL can be distributed to various sites, including the arcuate nuclei, which is adjacent to the third ventricle. The hypothalamus is also capable of *de novo* synthesis of PRL, a process that is regulated by an estrogen-sensitive mechanism (135). Although locally produced PRL may act as a mitogen for astrocytes (139), its participation in the short loop feedback on the TIDA neurons is doubtful.

The PRL-R belongs to the hematopoietic receptor family that includes GH, many cytokines, and some growth factors (for review, see Refs. 140–142). These are characterized by a single hydrophobic transmembrane domain that divides the receptor into an extracellular ligand binding domain and an intracellular domain. Features common to the extracellular domain include four paired cysteine residues and a wsxws (or WS) motif (tryptophan-serine-any amino acid-tryptophan-serine) that are involved in the formation of a ligand binding pocket. The cytoplasmic domains of the receptors differ in size and structure among the various family members. A hydrophobic proline-rich motif (homology box 1), located near the transmembrane region, is essential for signal transduction of all ligands studied. The PRL-R and several other hematopoietic receptors also contain a less-conserved cytoplasmic region, denoted box 2, whose function is not as well defined (143).

In the rat, alternative splicing generates two PRL-R isoforms, a short isoform of 291 amino acids and a long isoform of 591 amino acids. They have identical extracellular domains but differ in the length and sequence of the intracellular domain (Fig. 4). The promoter of the PRL-R gene belongs to a TATA-less/noninitiator class and has at least three regions that direct transcription from alternative sites in a tissue-specific manner (142). Both long and short receptor isoforms are expressed in most tissues, and their ratio is altered under many conditions (144). Although the exact function of each isoform remains to be fully defined, their coupling to different signal transduction pathways accounts for many of the pleiotropic actions of PRL (143). An "intermediate" form, lacking 198 amino acids due to a deletion, is uniquely expressed by the Nb2 rat T lymphocyte cell line (145) and confers growth dependence on PRL by these cells. A soluble form of the receptor, named PRL binding protein, resulting either from alternative splicing of the transcript or

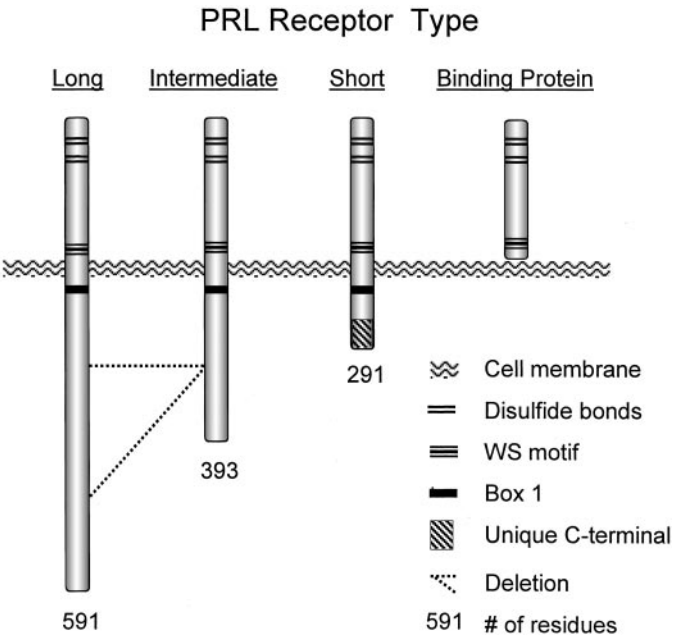

FIG. 4. Diagram of the different types of PRL-Rs in the rat. The PRL-R belongs to the hematopoietic receptor family characterized by a single short TMD. The extracellular ligand binding domain has several common features, including two disulfide bonds and WS (Trp-Ser-X-Trp-Ser) motif. A proline-rich homology box 1, located near the transmembrane region, is essential for signal transduction. The long and short isoforms are the products of differential splicing, with the short isoform possessing a distinct C terminus. An intermediate isoform, unique to Nb2 lymphocytes, has a deletion of 198 amino acids. A soluble form of the receptor, termed "PRL binding protein," has also been detected. [Derived from C. Bole-Feysot *et al.*: *Endocr Rev* 19: 225–268, 1998 (140). © The Endocrine Society.]

proteolytic cleavage of a membrane-bound receptor protein, has also been detected (146). The human PRL-R is encoded by a single gene that contains at least 10 exons and is located on chromosome 5 in close proximity to the GH receptor gene (147). In addition to the long form, a truncated isoform was identified in human breast cancer cell lines (148).

Similar to the GH receptor, binding of PRL to its receptor induces receptor dimerization (149). This engages two independent binding sites on the PRL molecule: site 1 composed of helices 1 and 4, and site 2 composed of helices 1 and 3 (140). At high concentrations, PRL can saturate the receptor and hinders further receptor dimerization, explaining the often observed bell-shaped, dose-dependent curves. In some cells, receptor-bound PRL is rapidly internalized and may be translocated to the nucleus (150). Intranuclear accumulation of PRL is stimulated by IL-2, is maximal within 6 h of treatment, and is inhibited by extracellular anti-PRL antibodies. However, the presence of intranuclear PRL and its specific functions are controversial (151). In the choroid plexus (152), amniochorion (153), and mammary epithelial cells (154), a PRL-R/binding protein, possibly of a different structure, acts as a transporter that translocates PRL from blood to the respective fluid compartments, *i.e.*, CSF, amniotic fluid, and milk.

Unlike many growth factor receptors, the PRL-R has no endogenous kinase activity, using instead the Janus kinase (JAK)-signal transducer and activator of transcription (Stat)

pathway as its major signal transduction mechanism (for reviews, see Refs.140 and 155). JAK2, which is constitutively associated with the PRL-R, is phosphorylated upon receptor activation by the ligand. In turn, the activated JAK2 phosphorylates other proteins, including the receptor itself and Stat proteins, *e.g.*, Stat 1, Stat 3, and Stat 5 (a and b isoforms). The phosphorylated Stat proteins dimerize and translocate to the nucleus where they bind to specific DNA motifs, called γ-interferon-activated sites, in the promoter regions of PRL target genes. Other transduction pathways, including the MAPK cascade and Src kinases, have been implicated in PRL-R signal transduction (reviewed in Ref. 140).

The mRNA of the PRL-R is expressed in practically all tissues with the highest density seen in the liver, choroid plexus, ovary, and mammary gland (144). Next to the choroid plexus, the hypothalamus has the highest density of PRL-R in the brain, with a dissociation constant (K_d) of 0.2–0.3 nM and maximal binding (B_{max}) of 5–10 fmol/mg proteins (156). Many hypothalamic nuclei, including the rostral arcuate and periventricular nuclei, in which perikarya of the TIDA and PHDA neurons are located, express mRNA and contain immunoreactive PRL-R (157). However, the receptor is expressed only by some, but not all, TH-positive neurons (98, 157). The long isoform is predominant, but both isoforms appear to be regulated under some conditions (158).

The expression of hypothalamic PRL-R mRNA is altered under several physiological conditions without a clear delineation of the specific hormones involved. Expression of the long isoform is increased during proestrus, the second half of pregnancy, and in aged female rats (159–161). Alterations in circulating PRL levels during lactation (158) and after hypophysectomy (156) increase and decrease, respectively, the hypothalamic PRL-R mRNA levels, suggesting that PRL regulates its own receptors. Similarly, chronic treatment of OVEX rats with estrogens resulted in increased expression of PRL-R mRNA in the TIDA/THDA neurons, paralleling the rise in plasma PRL levels (157). This effect was most pronounced in the dorsomedial and rostral arcuate nuclei and was attenuated by cotreatment with progesterone.

Changes in hypothalamic PRL-R mRNA expression are accompanied by an increased level of receptor protein. For example, immunoreactive PRL-R is higher in the medial preoptic area and periventricular and arcuate nuclei during lactation than in diestrus (162). During lactation, the PRL-R protein becomes detectable in several sites, such as the lateral hypothalamic, supraoptic, paraventricular, suprachiasmatic, and ventromedial nuclei that are undetectable during diestrus. The long isoform is induced in both female and male rats sensitized to pups and exhibiting maternal behaviors (159, 160). In females, receptor induction is dependent on intact ovaries and pituitary because no changes are observed in OVEX or hypophysectomized females when maternal sensitization occurs. In males, this induction is facilitated by PRL administration and is suppressed by cohabitation with females.

TH is one of the targets of PRL action within the hypothalamus, and both short-term enzyme activation (107, 163) and long-term enzyme induction (164, 165) have been observed. PRL immunoneutralization reverses the estrogen-induced increase in dopamine turnover in the median eminence and the intermediate lobe, albeit at different times during the PRL surge (166). In contrast, the observed effect of PRL immunoneutralization on the THDA system is minimal. Another laboratory found that PRL antiserum suppressed basal and haloperidol-induced increases in TIDA activity within 1–2 h, establishing a rapid action of PRL (167). This feedback mechanism is operational in both males and cycling females but is attenuated during late lactation, commensurate with the requirement for prolonged maintenance of elevated PRL levels during this time (168). Chronic hypoprolactinemia and hyperprolactinemia reduce and augment, respectively, TH mRNA levels in the arcuate nucleus but not in the substantia nigra, establishing the site specificity of enzyme induction (107).

PRL also exerts tropic effects on dopaminergic neuronal differentiation, best illustrated in animals with inherited PRL deficiency. Spontaneous mutations of the Snell and Ames dwarf mice result in the absence of lactotrophs, somatotrophs, and thyrotrophs and their respective hormones. The Snell mouse has mutations in the Pit-1 gene (169), a pituitary-specific transcription factor essential for differentiation and maintenance of the three pituitary cell types. The Ames mouse has a mutation in the Prop-1 gene, which normally activates Pit-1, leaving the animals with residual numbers of lactotrophs and somatotrophs (170). Homozygous dwarfs are growth retarded, sterile, and unable to lactate. Dopamine levels are severely depressed in both the TIDA and THDA systems, but not in other brain regions, with no change in norepinephrine (171).

Notably, the TIDA neurons do not differ between dwarfs and normal siblings until d 21 of age. In wild-type mice, dopamine levels and TH-positive neurons continue to increase, whereas those in the dwarfs are unchanged or decline (reviewed in Ref. 172). The dopamine deficiency can be reversed by PRL therapy, but only if initiated at an early postnatal period; adult dwarfs are refractory to PRL. This indicates that PRL acts as a neurotropic factor for the TIDA neurons during a specific developmental window of time. An unresolved issue is whether early postnatal TIDA development is totally independent of PRL or is supported by PRL that is provided by the maternal milk (173).

PRL functions as both a mitogen and survival factor in mammary and immune cells (for review, see Refs. 174–176), and may have similar functions in the brain. Astrocytes are the most numerous cells in the brain. Unlike neurons, they are capable of proliferation in adulthood. Astrocytes often function as immunocompetent cells and react to brain injury by increased proliferation, cytokine release, and antigen presentation. There is evidence that PRL, but not GH, induces proliferation of growth-arrested astrocytes. The mitogenic effects of PRL require serum-derived factors, suggesting that it functions as a coactivator (139). PRL temporally increases expression of tumor necrosis factor-α, IL-1α, and TGF-α in astrocytes, which in turn may synergize with PRL in promoting astrocyte proliferation and secretory activity (177). The mitogenic/secretory effects of PRL appear to be mediated by the JAK2/Stat pathway, providing the first evidence that PRL signaling within the brain is the same as in peripheral tissues (178, 179). It remains to be determined whether

the action of PRL on astrocytes directly or indirectly impinges on the TIDA neurons.

D. Effects of ovarian steroids

Estrogens affect PRL homeostasis at several anatomic sites that include the hypothalamus, posterior pituitary, and anterior pituitary (for review, see Ref. 105). The importance of estrogens in the control of the TIDA neurons is underscored by the higher basal activity of TIDA neurons in females, the suppression of neuronal activity by ovariectomy, and its reversal by estrogen. Some, but not all, of these actions are believed to be mediated by estrogen-induced PRL secretion. Exposure of fetal hypothalamic neuronal cultures to estrogen suppressed basal TH activity and reduced TH mRNA levels, indicating an estrogenic action that is not mediated by PRL (180). On the other hand, *in vivo* experiments often yield conflicting results because prolonged exposure and high doses of estrogens invariably increase PRL, thereby activating the PRL short loop feedback mechanism. Estrogen alone does not alter TH expression in the arcuate nucleus of monkeys, but causes significant decreases when followed by progesterone (181).

The effects of estrogens are mediated by two receptors, ERα and ERβ, that are the products of different genes (see Fig. 5). The ER is a member of the ligand-activated nuclear receptor gene superfamily, which includes other steroid receptors, receptors for thyroid hormones, and retinoic acid (for reviews, see Refs. 182–184). The ERα gene is 140-kb long with 8 exons and large introns. The ERα protein, composed of 595 amino acids, is divided into five functional domains: a DNA binding domain (DBD), a hormone binding domain (HBD), two transcriptional activation domains [activation function (AF)-1 and AF-2] and a hinge region. The unoccupied receptor is located in the nucleus in an inactive form associated with the molecular chaperone heat shock protein 90. Ligand binding activates the receptor by dissociating heat shock protein 90 and enabling receptor dimerization (185). The HBD is organized in several α-helices that form a ligand binding pocket (186), whereas the DBD folds into two dissimilar zinc fingers that interact with an estrogen response element (ERE) on target genes (187). Both domains are required for receptor dimerization.

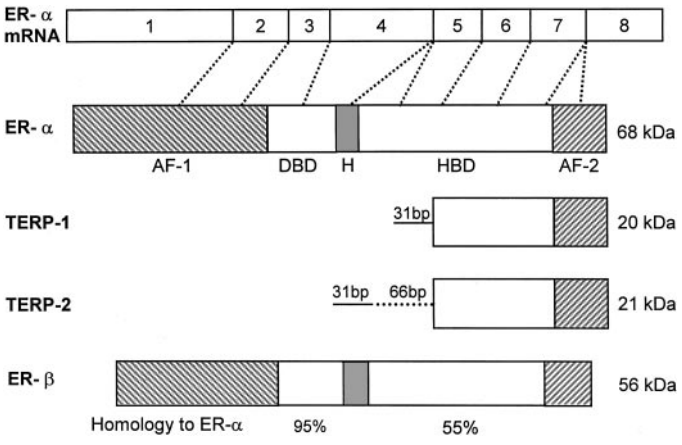

FIG. 5. Diagram of ERs. ERα and ERβ are the products of different genes. The ERα transcript is made of 8 exons and encodes a 68-kDa protein, which is divided into several functional domains: AF-1, DBD, hinge region (H), HBD, and AF-2. The pituitary also expresses TERP-1 and TERP-2, both of which are missing the first 4 exons and have unique stretches of 31 bp with an additional 66 bp in TERP-2. ERβ is a smaller protein than ERα (56–58 kDa) with a 95% homology to ERα in the DBD and 55% homology in the HBD but little homology in the AF domains. Several ERβ isoforms have also been identified. The different receptors can form either homodimers or heterodimers.

Transcriptional activation of ER involves both AF-1 and AF-2. AF-1 is a ligand-independent domain located in the N terminus, whereas the ligand-dependent AF-2 is near the C terminus, partially overlapping with the HBD (188). The two domains can act in synergy, but the contribution of each to transcriptional activity varies in a promoter- and cell-specific manner. After binding to DNA, the activated ER recruits coactivator proteins and forms a transcription complex that interacts with basal transcription factors that mediate RNA polymerase II-dependent transcription. Some coactivators are shared with several steroid receptors, whereas others are unique to the ER; several corepressors have also been identified (reviewed in Ref. 189). A consensus ERE, based on the *Xenopus* vitellogenin gene, is composed of a 13-oligomer palindrome, but sequences may differ by 1 or more nucleotides (190).

The ERβ protein, comprised of 485 amino acids, is smaller than ERα (for review, see Refs. 184 and 191). The two receptors share 95% homology in the DBD, 60% in the HBD, but less than 25% in AF-1 (Fig. 5). ERβ has a similar binding affinity for E2 to ERα, but a higher affinity for the antiestrogen 4-OH-tamoxifen and several xenoestrogens. Splice variants of ERβ have insertions or deletions in the HBD that alter the binding affinity of some estrogenic ligands, including xenoestrogens (192). The ERβ protein also binds to the consensus ERE and can form either homodimers or heterodimers with ERα (193). The tissue distribution of ERβ differs from that of ERα, with a strong expression in the ovary, prostate, lung, and brain. Within the hypothalamus, ERβ expression is especially high in oxytocin- and vasopressin-expressing magnocellular neurons but is only weakly expressed in the dorsal arcuate nucleus (194).

In rat embryos, ERβ is detectable in the pituitary anlage as early as ED 12–13, whereas ERα becomes detectable only on ED 17 (195). In adult rats, ERβ is expressed in most pituitary cell types (195), including lactotrophs (196), but is undetectable in the mouse pituitary (197). The rat pituitary also expresses truncated ER products (TERPs; see Fig. 5), which have not been detected in other tissues (198). Expression of TERPs is up-regulated by estrogens (196), and although they lack DBD, they can dimerize with either ERα or ERβ, thereby forming inactive dimers that may compete with the wild-type receptors for coactivators (198).

Only a fraction of TH-positive neurons in the arcuate nucleus express ERs with a predominance of ERα (199). Similar to PRL, it is unclear whether the estrogenic effect on the TIDA neurons is direct or is mediated by other neurotransmitters or neuropeptides. Many studies have attempted to delineate the temporal effects of ovarian steroids on the dopaminergic neurons throughout the estrous cycles. The low circulating PRL levels observed during most of the cycle rise abruptly on the afternoon of proestrus. Unlike the LH surge, which is sharp and symmetrical, the PRL surge is composed of three

distinct phases: an early sharp peak, a prolonged plateau, and a termination phase. Dopamine turnover rates (200, 201) and TH activity (106) are higher during the peak phase than during the plateau, increasing again during the termination phase. Acute ovariectomy on the morning of proestrus prevents the decline in TH activity and mRNA levels (202, 203), with progesterone, but not E2 replacement, restoring TH function. These and several other studies (121, 122, 163) suggest that although a decrease in TIDA neuronal activity contributes to the proestrus PRL surge, PRFs must be involved.

Xenoestrogens are synthetic chemicals with little structural similarity to E2 that can bind to the ER and mimic or interfere with the actions of endogenous estrogens (reviewed in Refs. 204–206). Whereas most studies focused on the effects of xenoestrogens on the reproductive tracts, several investigators examined their action on the neuroendocrine axis. For example, bisphenol A (BPA), a monomer of polycarbonate plastics and epoxy resins, and octylphenol (OP), a common constituent of detergents and herbicides, increase PRL gene expression and release and induce cell proliferation in cultured lactotrophs (207–209). Treatment of Fischer 344, but not Sprague Dawley, female rats with moderate doses of BPA resulted in hyperprolactinemia (207); unlike the effect of E2, pituitary weight did not increase. These results suggest genetic predisposition to the action of BPA and its partial agonist activity.

Recently, exposure of newborn male and female rats to either BPA or OP caused a delayed and prolonged hyperprolactinemia and alterations in ER expression in both the hypothalamus and anterior pituitary (210). As shown in Fig. 6, 1 month after treatment of newborn rats with OP, serum PRL levels were elevated, whereas hypothalamic TH activity was markedly suppressed. This suggests that the xenoestrogen caused prolonged down-regulation of TH expression or activity and likely impaired its ability to respond to short loop feedback effect by elevated PRL.

Estrogens have long been viewed as ligands that bind to nuclear receptors and function as regulators of transcription. On certain cells, however, estrogens exert very rapid actions (seconds to minutes) that cannot be explained by classical genomic mechanisms. The best documented fast action of estrogens is on endothelial cells, where they rapidly stimulate nitric oxide (NO) release, activate endothelial NO synthase (eNOS), and induce its translocation from the plasma membrane to intracellular sites by a calcium-dependent mechanism (211–214).

Cross-talk between estrogen-induced neuropeptide release and endothelial NO has also been reported, showing a rapid increase in GnRH release from the median eminence by BSA-conjugated E2 that cannot pass through the plasma membrane (215). More importantly, this response may be mediated by endothelial-derived NO because it was abolished by NO scavengers and specific eNOS inhibitors. Recently, injection of NO inhibitors or intracerebral administration of NOS antisense oligonucleotides blocked the proestrus PRL surge and partially reversed the decline in TIDA activity, suggesting that NO plays a role on the estrogen-induced changes in dopaminergic neuronal activity (120).

Rapid, nongenomic action of estrogens has also been observed in neurons. An early study reported that 17β-E2 stimulated dopamine release from depolarized posterior pituitary neurons within 20 min, whereas 17α-E2 and T were ineffective (216). Short-term and stereospecific inhibitory effects of E2 on TH activity was later reported using hypothalamic slices (108). E2 also enhanced the amplitude of

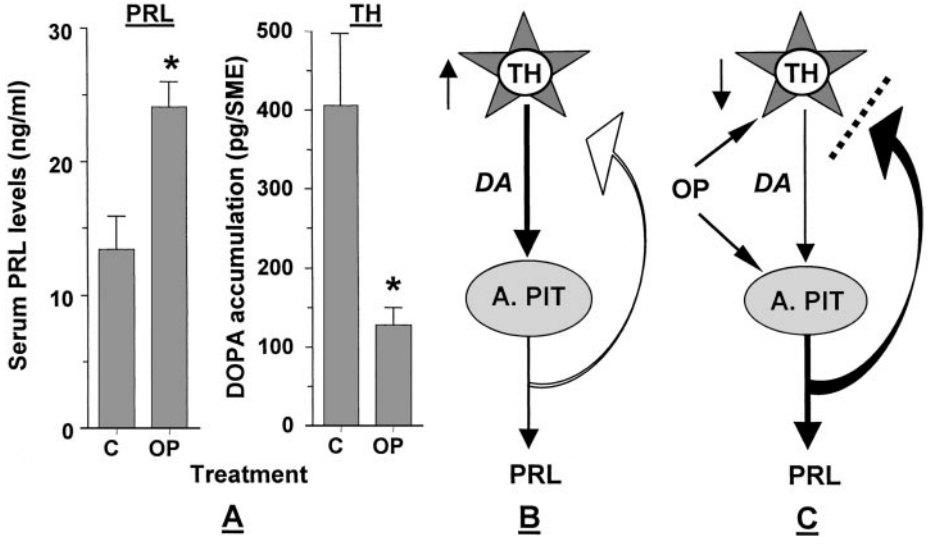

FIG. 6. Long-term effects of the xenoestrogen OP on PRL release and TH activity. Panel A, Newborn female rats were treated with OP (100 μg/day) or vehicle (control) for the first 5 d of life. On d 30, rats were killed and serum PRL levels were determined by RIA. The dissected stalk median eminence (SME) were incubated for 30 min with NSD-1015, a DDC inhibitor, and DOPA accumulation was determined by HPLC (S. Khurana and N. Ben-Jonathan, unpublished observations). Panel B, Diagram of the relationship between TH activity, dopamine (DA) release, and serum PRL levels under normal conditions. An active TH maintains high DA input, which suppresses PRL release. Panel C, Postulated mode of action of OP at both the hypothalamic and pituitary sites. OP binds to ERs on the lactotrophs and increases PRL secretion; it may also alter the number or activity of lactotrophs. Despite elevated serum PRL levels, there is an ineffective feedback by PRL because DA output is reduced. This could be due to altered expression/activity of TH, down-regulation of the PRL-R on the TIDA neurons, or both.

kainate-induced currents in dispersed hippocampal neurons (217). This effect was also seen in neurons from ERα-knockout mice and in the presence of ICI 182,780 (which blocks both ERα and ERβ), suggesting distinct estrogen binding sites. In both neurons and glia, the fast estrogenic action is often mediated by ERK1/2 phosphorylation via the MAPK signaling pathway (218). Because ERK was activated by BSA-conjugated E2 in the latter study, a membrane ER was implicated.

Recently, rapid (within 5–6 min) induction of PRL release from GH3 cells by a low dose of E2 has been reported (219). In addition, E2 rapidly activates ERK1/2 in pituitary lactotrophs, whereas blocking the MAPK signal transduction pathway ablated the estrogen-induced PRL gene expression (220). The nature of the putative membrane ER is controversial. Although its identity in neurons remains elusive, studies using ER antagonists and antibodies suggest that ERα might be the putative membrane receptor in endothelial (221) and GH3 (222) cells. A recent report localized ERα in bovine aortic endothelial cells to caveolae (223). Caveolae are pleomorphic membrane microdomains with distinct phospholipid components that are enriched in various receptors and signaling molecules (reviewed in Ref. 224). In both endothelia and cardiac myocytes, eNOS is contained within caveolae and is tonically inhibited by caveolin-1. The localization of ER within caveolae provides a plausible explanation for its sequestration within the plasma membrane and the rapid activation of eNOS. Details of such interactions and how ER might be routed to two distinct compartments, nucleus and caveolae, are yet to be determined.

E. Interactions with neuropeptides and neurotransmitters

In addition to PRL and estrogens, the TIDA/THDA neurons interact with a number of neuropeptides and neurotransmitters, as illustrated schematically in Fig. 7. Only selected factors will be discussed here to exemplify the complexity of the dopaminergic system. Opioid peptides stimulate PRL release by inhibiting the TIDA neurons. The hypothalamus contains perikarya of the three opioid classes, *i.e.*, endorphins, dynorphins, and enkephalins, all of which participate in the regulation of PRL release under some conditions. The opioid peptides exert their effect via three major receptor types, μ, κ, and δ, which belong to the superfamily of seven transmembrane G protein-coupled receptors (for review, see Ref. 225).

Although the arcuate nucleus contains pro-opiomelanocortin (POMC)-positive cells, only a minority of the TIDA perikarya receive contacts from β-endorphin terminals, and most interactions occur at their median eminence terminals (226), likely through local μ-receptors (227). On the other hand, most TIDA neurons have synaptic contacts with dynorphin terminals (228), which also innervate the intermediate lobe of the pituitary (229). Indeed, opioid peptides belonging to the proenkephalin and prodynorphin families may act as paracrine regulators of the TIDA (230) as well as THDA neurons (216, 231).

The recognition that endogenous opioids inhibit the TIDA neurons is based on many studies with receptor agonists. Early studies employing systemic and intraventricular administration of the opioid agonist morphine reported lowered TIDA neuronal activity and reduced dopamine levels in portal blood. Conversely, central administration of either μ- or κ-receptor antagonists increase TIDA neuronal activity (reviewed in Refs. 105 and 113). Several lines of evidence support direct action of the opioid peptides on the TIDA neurons. One is activation of potassium conductance and hyperpolarization of dopaminergic neurons by a μ-opioid agonist (232), and another is inhibition of the firing rates of arcuate nucleus neurons in hypothalamic slices by all three classes of opioid peptides (233). A third line of evidence is the reported inhibition of dopamine release and synthesis from incubated median eminence by β-endorphin (231) and enkephalin (234).

Interactions between opioid peptides and TH-positive neurons are especially prominent during the proestrus PRL surge (235), the nocturnal PRL rise in pregnant rats (236), and during suckling (230, 237). Stress-induced PRL release is also mediated, at least in part, by disinhibition of endogenous opioids' control over the TIDA neurons (238). Most studies to date have used naloxone, a broad-spectrum opioid recep-

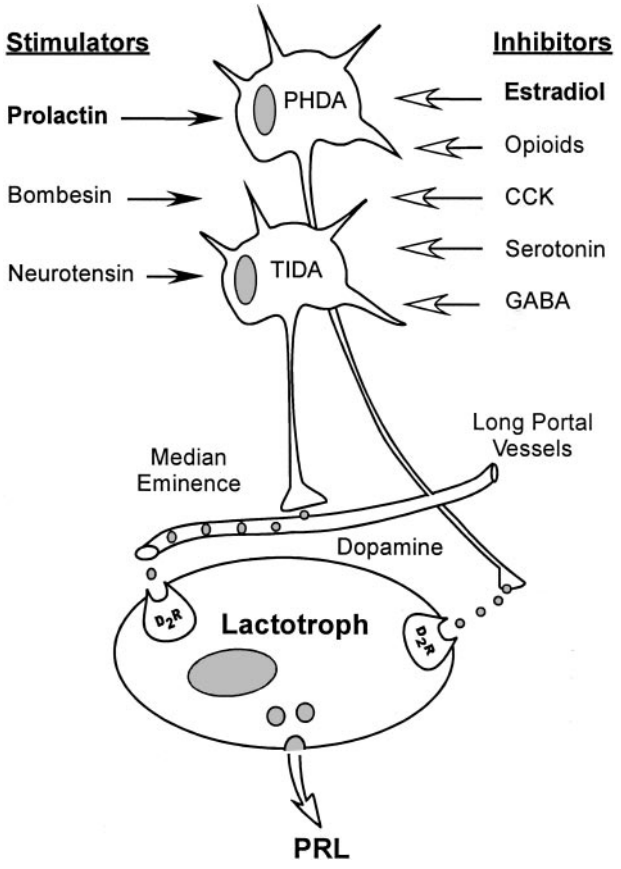

FIG. 7. Selected compounds that act as stimulators or inhibitors of the hypothalamic dopaminergic neurons. PRL and E2 function as the primary stimulators and inhibitors, respectively, of the dopaminergic neurons. Bombesin and neurotensin stimulate TH activity, whereas opioid peptides, CCK, serotonin, and GABA act as inhibitors. This is only a partial list, and several of these compounds have a dual action on PRL release, depending upon their sites of action. Specific actions via the TIDA or THDA neurons cannot be assigned with certainty. [Derived from M. E. Freeman *et al.*: *Physiol Rev* 80:1523:1631, 2000 (105)].

tor antagonist. For example, infusion of naloxone to lactating rats during suckling resulted in a marked suppression of circulating PRL levels and a concomitant increase in TIDA neurons' activity and TH expression. Such alterations occur in both the arcuate nucleus and median eminence but not in the zona incerta, establishing site specificity (237). Pups of the naloxone-treated mothers failed to gain weight, indicating lower milk availability and perhaps some inhibition of oxytocin release. As mentioned before, the TIDA neurons in males are under tonic inhibition by endogenous opioids peptides through κ-receptors (130). These data, supported by immunoneutralization studies (230), indicate that the endogenous opioids comprise an integral component of the system that generates PRL surges, but they do not rule out their potential interactions with nondopaminergic neurons that produce PRL releasing factors.

Cholecystokinin (CCK), produced in several molecular forms from a larger precursor protein, also increases PRL release indirectly. Originally discovered in the gut, CCK is widely distributed in the brain, where it participates in the regulation of appetite and body temperature (reviewed in Ref. 239). CCK-8, the predominant form in the brain, exerts its actions mainly through the CCK-B receptor. Both immunoreactive CCK and its receptors are highly expressed in both the arcuate nucleus and the neural lobe (240). Third-ventricular administration of CCK stimulates PRL release (241), but its mode of action is complicated by gender differences, with males responding to a central CCK antagonist with increases, but OVEX rats with decreases, in PRL release (242). CCK has a robust stimulatory effect on the firing rates of arcuate nucleus neurons (243), but because this presumably increases dopamine release, it is not consistent with an overall stimulatory effect of CCK on PRL secretion.

Other neuropeptides, *e.g.*, bombesin, neurotensin, and NPY, inhibit PRL release by stimulating the TIDA neurons (for review, see Ref. 105). Their action, however, is often confounded by their ability to exert opposite effects on the pituitary itself. Bombesin is a 14-amino acid peptide isolated from the skin of a frog. Family members include gastrin-related peptide (GRP), neuromedins B and C, and ranatensin. Although there are many GRP-positive perikarya in the paraventricular and periventricular nuclei with projections to the SCN, there is little evidence that they innervate the arcuate nucleus or median eminence (244). Yet, these compounds have a potent stimulatory effect on unit activity of most arcuate neurons, an effect that is blocked by GRP receptor inhibitors (245). Also, intraventricular administration of bombesin-like peptides increases TIDA and PHDA activities in males, without affecting nigrostriatal or THDA neurons, and suppresses both PRL and α-MSH release (115). Bombesin-like peptides may also play a role in the circadian rhythm of PRL release, acting via the SCN (246). Further evidence for a role of endogenous GRP-related peptides in the control of PRL release is provided by the rise in serum PRL after central immunoneutralization of these peptides (247).

Neurotensin is a 13-amino acid peptide generated by post-translational processing of a large precursor protein (for review, see Ref. 248). It shows extensive colocalization with TIDA/PHDA neurons that project to the external layer of the median eminence and neurointermediate lobe (249). Like many neuropeptides, it exerts a dual action on PRL: central inhibition and peripheral stimulation (250). Neurotensin activates both the TIDA and THDA neurons and induces concomitant decreases in serum PRL and α-MSH levels (251). Indirect evidence suggests that it mediates the hyperprolactinemia-induced activation of TIDA neurons in both males and females (252). The effects of NPY, the most abundant neuropeptide in the brain, on PRL release are similar to those of neurotensin except that it does not mediate the PRL feedback on the TIDA neurons (for review, see Ref. 105).

Among neurotransmitters, the stimulatory effect of serotonin on PRL release has long been recognized (reviewed in Ref. 253). Serotonergic neurons originating from the raphe nucleus terminate in many hypothalamic nuclei including the SCN, paraventricular nuclei, and arcuate nuclei with projections into the intermediate lobe (254). Early studies using drugs that enhance serotonergic function, *i.e.*, precursors, receptor agonists, and uptake inhibitors, revealed transient rises in PRL release in rodents, but were less effective in humans. Conversely, drugs that impair serotonergic neurotransmission or lesions of the raphe nucleus and its projections prevented rises in PRL release in response to stress and suckling (for review, see Ref. 1). The stimulatory action of serotonin on PRL involves the 5-HT_{2A}, 5-HT_{2C}, and 5-HT_3 receptor subtypes (255). Although serotonin administration reduces dopamine levels in hypophysial portal blood (256) and suppresses TH activity (257), a consistent effect of serotonin agonists on TIDA neuronal activity has not been observed. Instead, the current dogma is that serotonin affects PRL secretion primarily by stimulating the release of PRF. The latter is supported by the increased PRL release from anterior pituitaries cocultured with posterior pituitaries in the presence of serotonin (258).

GABA, acting through GABA(A) receptors, appears to play a role in the control of basal and diurnal changes of TIDA neuronal activity, and contributes to increased PRL secretion (259). The participation of the cholinergic system in the regulation of PRL release is controversial, and the observed effects vary with the site of administration, the dose, and the physiological conditions of the animals. There is an endogenous rhythm of cholinergic neurons, which may contribute to the circadian changes that occur in both the TIDA neuronal activity and PRL release, but whether the cholinergic output is on the opioidergic or TIDA neurons remains to be determined (113). Histamine can also be added to the list of centrally and peripherally acting neurotransmitters with an unclear mechanism of action (105, 260).

IV. Dopamine and the Pituitary Lactotrophs

A. Dopamine and its receptor

Because the anterior pituitary is a non-neuronal tissue, the presence of dopamine in this lobe (121, 122), in association with PRL-containing secretory granules (261–263), raises the question as to its cellular origin. One possibility is *de novo* dopamine synthesis. The long-held dogma that the anterior pituitary is not innervated has been challenged by recent studies using sensitive staining and electron microscopy

techniques (reviewed in Ref. 264). The presence of few TH-positive, DBH-negative fibers in the rat anterior pituitary has been reported (265), although this has not been confirmed by others (111). TH mRNA was detected in the female rat anterior pituitary by RT-PCR and *in situ* hybridization (266), but a subsequent study found two alternatively spliced TH transcripts that do not encode a full-size TH protein (267). The fact that TH in the anterior pituitary may be nonfunctional, together with the lack of evidence for *de novo* synthesis, argue against local dopamine synthesis. On the other hand, TH activity and dopamine production were detected in anterior pituitaries grafted under the kidney capsule (268), and low levels of TH protein, similar in size to that in PC12 cells, were observed by Western blotting in GH3 cells (269). Therefore, a functional TH may be produced only under abnormal conditions such as in ectopic pituitaries, adenomas, or transformed lactotrophs.

Uptake of dopamine, brought to the pituitary by portal blood, is another conceivable source of dopamine within the anterior pituitary. If this is the case, either DAT or another dopamine transporter must be involved. DAT was detected in the rat anterior pituitary by antibodies raised against a synthetic peptide (270), but this was not confirmed by others (111). Indeed, expression of DAT in the anterior pituitary represents an unusual situation because the distribution of this transporter may be restricted to the CNS, where it always colocalizes with TH (34). However, dopamine reuptake also occurs in a Na^+-dependent manner in glial cells that do not express DAT, suggesting an alternate mechanism (271). GH3 cells were shown to take up MPP^+, a selective dopaminergic neurotoxin that generates Parkinson's-like symptoms (269). This uptake occurs in a concentration- dependent manner and is blocked by nomifensin, a selective dopamine reuptake inhibitor. Further exploration of dopamine uptake in the anterior pituitary, especially by lactotrophs, should provide an appealing mechanism for a local control of dopamine availability to these cells.

The predominant dopamine receptor in the pituitary, abundant in both lactotrophs and melanotrophs, is the D_2-type (73, 272). The D_2R, initially cloned from the rat brain, consists of 1245 nucleotides encoding a 415-amino acid protein (63). Subsequent studies identified a second cDNA with an 87-nucleotide insertion that encodes a 444-amino acid protein (273). Both sequences originate from a single gene spanning approximately 50 kb and containing 8 exons, with exon 1 encoding an untranslated sequence. Alternative splicing removes exon 6 and generates long and short isoforms (D_2L and D_2S, respectively), which differ in 29 amino acids in the third cytoplasmic loop that is involved in coupling to G proteins (see Fig. 8). The isoforms exhibit similar pharmacological properties and are coexpressed in the same cells, but because of select coupling to G proteins, they may serve different functions. The long isoform is predominant in the rat pituitary, striatum, and olfactory tubercle, whereas the short isoform is more abundant in the hypothalamus and the substantia nigra (274). The long isoform also predominates in normal human pituitaries (275). Activation of either isoform in rat lactotrophs mediates dopamine suppression of the PRL gene (276), with both isoforms inhibiting adenylyl cyclase but only the D_2S negatively coupled to the phospholipase signaling pathway (79, 277).

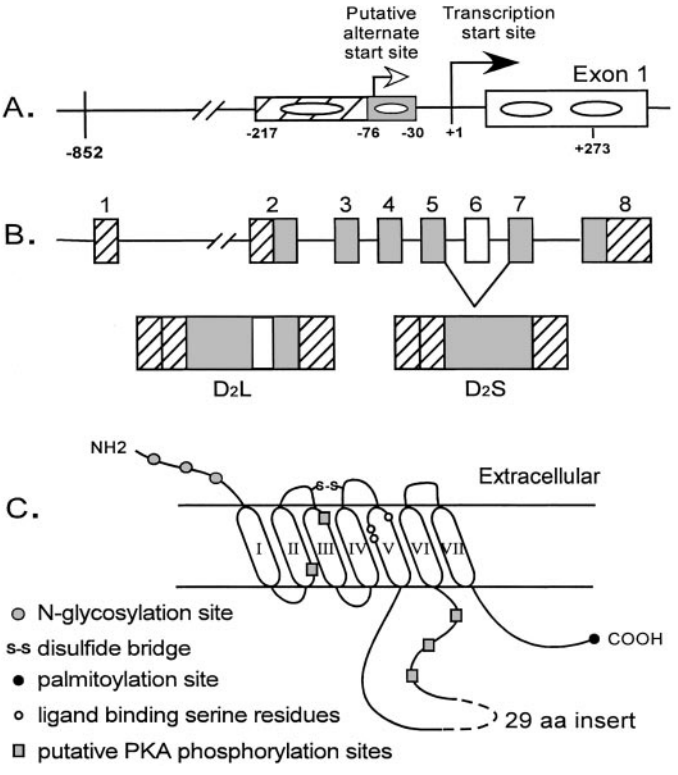

FIG. 8. Diagram of the D_2R. Panel A, The D_2R promoter region. The primary and additional putative transcription start sites are indicated by *solid and open arrows*, respectively. The promoter lacks TATA and CAAT boxes and has several putative Sp1 binding sites (*open ovals*). Negative and positive modulatory elements are designated by *hatched and dark rectangles*, respectively. Panel B, Organization of the D_2R gene and alternative splicing that generate the long (D_2L) and short (D_2S) isoforms. Exons, introns, and untranslated sequences are designated by *filled boxes*, *connecting lines*, and *hatched boxes*, respectively. The *empty box* is exon 6, which is absent in the D_2S transcript. Panel C, Diagram of the D_2R protein. Shown are the 7 TMDs and potential N-glycosylation, PKA phosphorylation, and palmitoylation sites. The disulfide bridge between extracellular loops and the serine residues involved in agonist binding in TMD V are also depicted. The 29-residue insert (*interrupted line*) in the large intracellular loop is specific to the long isoform. [Derived from R. Piceti *et al.*: *Crit Rev Neurobiol* 11:121–142, 1997 (78)].

Ovarian steroids can affect the ratio of D_2L/D_2S in the pituitary both *in vitro* and *in vivo*. In MMQ cells, a rat lactotroph cell line, estrogen increases the expression of D_2L, an induction that is reversed by progesterone. Because hormone treatment does not alter total receptor mRNA levels, the steroids may regulate the expression of a splicing factor favoring production of the long isoform (278). Castration of male rats increases the ratio of D_2L to D_2S in the pituitary, whereas T treatment reverses the effect of castration in the pituitary but not in the substantia nigra. Because E2 mimics this action in males, T may act after being aromatized to E2. In contrast, neither castration nor hormonal treatment modifies the splicing of the D_2R mRNA in the striatum (278). Long-term treatment of male rats with the D_2R antagonist haloperidol increases the expression of the short isoform in the anterior pituitary (279). An early study suggested a direct

effect of E2 on the number of anterior pituitary dopamine binding sites without a change in binding affinity (280). Such rapid, reversible, and stereospecific effect represents another example of the nongenomic action of estrogens.

The effects of dopamine agonists and antagonists on pituitary D_2R expression is controversial. In one study, haloperidol increased D_2R mRNA levels in the neurointermediate lobe, whereas bromocriptine caused no change, with neither compound changing D_2R mRNA levels in the anterior lobe (272). In another study, dopamine increased the expression of D_2R in primary dispersed rat anterior pituitary cells (281). Several growth factors also alter D_2R expression by the lactotrophs. For example, exposure of GH3 cells, which lack functional D_2R, to epidermal growth factor (EGF) induces the expression of both D_2R isoforms, which were coupled to potassium channels and responded to selective dopamine agonists (282). In addition, NGF induces D_2R expression in bromocriptine-resistant human prolactinomas grown in soft agar or in nude mice (283). Ablation of NGF production by antisense oligonucleotides in bromocriptine-responsive cells resulted in loss of D_2R expression and unresponsiveness to dopamine (284). NGF treatment also induces the expression of D_2R in GH3 cells (285). Unlike the effect of EGF, NGF induced the expression of dopamine receptors that were coupled to adenylyl cyclase rather than to potassium channels.

As shown in Fig. 8, the primary transcription initiation site of the rat D_2R gene is located 320 bp upstream from the first exon, with a minor site 70 bp further upstream (286). The promoter lacks TATA and CAAT boxes and is rich in G+C content with several putative Sp1 binding sites. Consensus sequences have also been identified for AP1, AP2, GATA, and the thyroid receptor/RAR. Yet the identity of specific promoter elements and binding proteins that regulate D_2R gene transcription is unclear (287, 288). A *cis*-acting element, located between −75 and −30 relative to the transcription start site, positively regulates the promoter, whereas negative regulation is exerted further upstream (287). The D_2R promoter has been ligated to reporter genes and transfected into several cell types. Promoter activity is robust in cell lines that normally express D_2R with only limited activity in cells that do not (78), indicating that cell-specific transcription factors are necessary for the regulation of the D_2R gene.

B. Actions and signal transduction

Although dopamine has been recognized as the physiological inhibitor of lactotrophs for over three decades, its exact mechanism of action is less understood than that of other hypothalamic regulating factors. The reasons for this are as follows: First, it is more difficult to study inhibition than stimulation. Second, lactotrophs are highly heterogeneous with respect to basal PRL release and responsiveness to dopamine. Third, there is a paucity of lactotroph cell lines with endogenous and functional dopamine receptors. Fourth, at very low concentrations, dopamine has been reported to stimulate, rather than inhibit, PRL secretion.

Lactotrophs *in vivo* are continuously exposed to dopamine. When cultured without dopamine, their cellular properties may be altered, deviating from the situation *in vivo*. Despite this caveat, many lines of evidence indicate that dopamine exerts diverse effects on the lactotrophs. This can be best illustrated when considered on a continuous time scale. Within seconds of exposure, dopamine induces membrane hyperpolarization leading to inactivation of voltage-gated calcium channels, reduced intracellular free calcium, and inhibition of PRL release from secretory granules (see Fig. 9). Within minutes to hours, dopamine suppresses adenylyl cyclase activity and lowers inositol phosphate metabolism, resulting in the suppression of PRL gene expression and changes in cell morphology. Within days, dopamine inhibits lactotroph proliferation and decreases the size of hypertrophied lactotrophs. It remains to be determined whether all these events occur within the same cell or involve different cell subpopulations and to what extent they progress sequentially or occur in parallel.

The rapidity by which dopamine inhibits PRL release suggests involvement of ion channels (for review, see Refs. 289 and 290). Application of whole-cell recording and patch clamp techniques established that lactotrophs are electrically excitable. Many lactotrophs exhibit spontaneous, voltage-dependent action potentials at frequencies between 0.2 and 0.5 Hz and a resting membrane potential of −40 to −60 mV (291). An interplay between tetrodotoxin-sensitive sodium current, T- and L-type calcium channels and calcium-dependent potassium channels contributes to the generation of this electrical activity. As judged by their electrical activity, lactotrophs are divided into two subpopulations: quiescent cells with a stable membrane potential that is insensitive to acute changes in extracellular calcium, and active cells with spon-

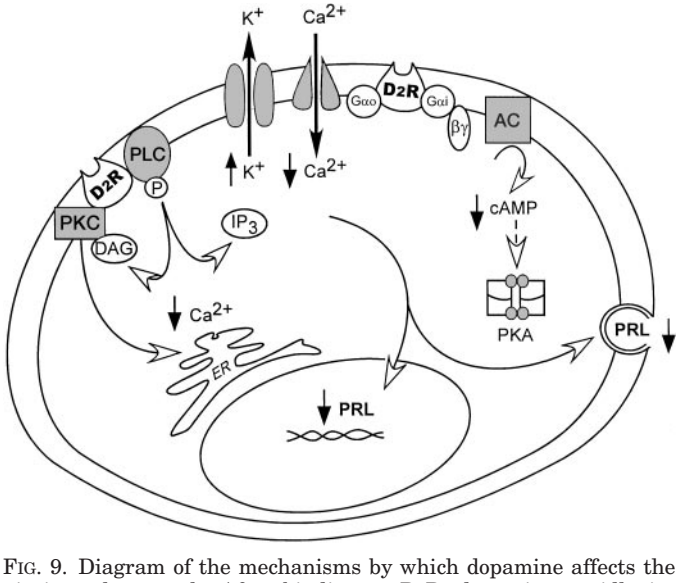

FIG. 9. Diagram of the mechanisms by which dopamine affects the pituitary lactotroph. After binding to D_2R, dopamine rapidly increases intracellular potassium and reduces calcium influx. This can be done by either a direct or an indirect coupling of the receptor via $G_\alpha 0$ to potassium channels, resulting in a decrease in calcium influx and an immediate suppression of PRL release. Further decreases in intracellular calcium are achieved by inhibition of PLC and PKC, resulting in reduced calcium mobilization from the endoplasmic reticulum (ER). The main mechanism for the suppression of PRL gene expression is done by coupling of the D_2R to $G_{\alpha i}$ proteins, resulting in inhibition of adenylyl cyclase (AC), decreases in cAMP production, and suppression of activated PKA.

taneous membrane depolarization and calcium oscillations that depend on extracellular calcium levels. The electrically active cells also form larger plaques in the reverse hemolytic plaque assay indicating higher basal PRL release (289).

Dopamine induces a very rapid fall in cytosolic free calcium concentration, particularly in spontaneously active lactotrophs (see Fig. 9). Two mechanisms have been proposed to explain this action. One is via coupling of the D_2R to voltage-gated calcium channels (292), which are substrates for phosphorylation/dephosphorylation by PKA and PKC (293, 294). However, the dopamine-induced reduction in cAMP levels is too slow to account for the speed with which calcium oscillations are suppressed by dopamine. Instead, it may reflect more prolonged changes in channel activity that is induced by dopamine. Another proposed mechanism is that dopamine reduces calcium influx indirectly, by evoking membrane hyperpolarization via inward rectifying current that increase potassium conductance (289). This results in the closing of voltage-gated calcium channels and a rapid decline in intracellular calcium. Because PRL release from storage granules is coupled to calcium availability, these processes explain the high basal PRL release by cultured lactotrophs in the absence of dopamine and its rapid suppression upon exposure to dopamine.

Several laboratories have reported that dopamine at extremely low concentrations (<0.1 nM) stimulates PRL release (295, 296). The stimulation is rather modest and has not been observed by all investigators. Several mechanisms have been proposed to explain this phenomenon, including predominance of the short dopamine receptor isoform (297), mediation by D_1 and D_5 receptors (298), involvement of $G_{i\alpha3}$ protein (299), and dopamine-induced increases in intracellular calcium by lactotroph subpopulations (296). Opponents of this notion argue that the observed stimulation can be explained by dopamine withdrawal, resulting in increased cytosolic free calcium concentration (291) and a rebound of PRL release that exceeds baseline levels. When the rebound occurs in the presence of secretagogues such as TRH or VIP, it results in a pronounced potentiation of their PRL-releasing actions (300). Presently, the issue of the stimulatory effect of dopamine is controversial and needs further assessment.

The primary mechanism by which dopamine suppresses PRL gene expression is by reducing intracellular cAMP levels (Fig. 9). Several studies have established that dopamine inhibits adenylyl cyclase activity in the anterior pituitary *in vivo* and in cultured lactotrophs *in vitro* (for review, see Ref. 131). The D_2R is coupled to pertussis toxin-sensitive signaling pathways, implicating its association with members of the $G_{i/o}$ protein family (301). Activation of the $G_{\alpha i}$- and $G_{\alpha o}$-subunits alter the activity of adenylyl cyclase types I, V, and VI (302). The $G\beta\gamma$-subunits, released by the activation of $G_{\alpha i/o}$, can enhance the activity of type II enzyme, enabling cross-talk between several biochemical pathways. The complex interaction of these pathways after D_2R activation alters cAMP levels and, consequently, PKA activity. PKA phosphorylates cytoplasmic and nuclear proteins and also regulates ion channel function and desensitization of G protein-coupled receptors. Increases in cAMP lead to functional activation of PKA by binding to the regulatory subunits and liberating the catalytic subunits, whereas a decrease in cAMP suppresses PKA by maintaining it in an inactive conformation. It is this suppression of PKA activity, especially in the presence of secretagogues, that mediates many of the cellular responses of lactotrophs to dopamine (303), with some evidence for the involvement of PKC as well (304).

The role of IP3 as a mediator of dopamine action on the lactotrophs is unclear (Fig. 9). Although activation of D_2R in pituitary lactotrophs results in inhibition of inositol phosphate production, this is a relatively late event that may be secondary to decreased calcium influx (305). In GH4C1 cells transfected with either D_2L or D_2S receptor isoforms, only the short isoform is negatively coupled to PLC, whereas both are negatively coupled to adenylyl cyclase (79). The short isoform can also activate PLD through a pertussis toxin-independent mechanism that may involve Rho proteins (277). The cellular response to D_2R activation likely depends on the available G proteins in any given cell. The existence of two receptor isoforms that are differentially coupled to various G proteins within the same cell likely serves to amplify and diversify the response to dopamine.

Other actions of dopamine on the lactotrophs have been reported. One is an indirect suppression of the PRL gene by inhibiting the expression of Pit-1, a pituitary-specific transcription factor that is necessary for GH and PRL gene expression (for review, see Ref. 306). Incubation of dopamine agonists with GH cells transfected with D_2R reversibly inhibits Pit-1 via a dopamine-sensitive domain in the N-terminal sequence of Pit-1 (307). Dopamine also induces cortical actin assembly and stabilization in lactotrophs by increasing the expression of the actin anchoring proteins talin and α-actinin and maintaining a rounded cell shape (308). Whether changes in cell shape, observed under *in vitro* conditions, are relevant to a physiological action of dopamine is unclear.

C. Antiproliferative activity

The best evidence supporting the antimitotic activity of dopamine on the lactotrophs comes from the induction of pituitary hyperplasia in D_2R-deficient mice and pituitary hypoplasia in DAT-deficient mice, discussed in *Section V.A*. In addition, bromocriptine, the dopamine receptor agonist, causes considerable shrinkage of human prolactinomas (discussed in *Section VI*). Here we will review evidence derived either from normal laboratory animals or from cultured cells.

Although dopamine is effective in suppressing proliferation of the melanotrophs during early postnatal development (101), a similar action of dopamine or its agonists on the developing lactotrophs has not been reported. The increase in the number of lactotrophs in females during puberty appears to be due to increased circulating levels of estrogens rather than a decreased dopaminergic tone (309). On the other hand, bromocriptine is effective in reversing the pituitary hyperplasia that is induced by exogenous estrogens in certain strains of rats, *e.g.*, Fischer 344 (310, 311). The estrogen-induced TGFα expression is rapidly attenuated by D_2R activation and is accompanied by regression of the estrogen-induced pituitary growth (312). Interaction between these opposing hormone/transmitter responses is responsible, at least in part, for the growth

potential of the anterior pituitary. Whether other estrogen-responsive genes that are involved in regulating pituitary proliferation such as TGFβ (313) are also affected by dopamine remains to be determined.

Cultured normal lactotrophs treated with dopamine or bromocriptine show decreased bromodeoxyuridine-labeling indices, whereas pretreatment with D_2R antagonists blocks this effect (314). The decrease in nuclear bromodeoxyuridine incorporation, reflecting a decrease in DNA replication, is consistent with an antimitotic activity of dopamine in the pituitary. Interestingly, dopamine and its agonists have neurotoxic effects on cultured neurons that can be prevented by free radical scavengers and antioxidants (315). Similar effects have been observed upon long-term incubation of GH3 cells with bromocriptine (316). However, because GH3 cells do not express functional D_2R, the cytotoxic action of bromocriptine likely occurs by a dopamine receptor-independent mechanism. Indeed, bromocriptine inhibits proliferation of several cell types, such as smooth muscle cells that do not express D_2R (317), and induces apoptosis by activating p38 MAPK in GH3 cells (318).

V. Lessons Learned from Transgenic Mice

A. Dopamine D_2 receptor (D_2R) and transporter

Two types of transgenic mice with altered dopamine have been generated: those with a deletion of D_2R, which prevents dopamine action, and those with a deletion of DAT, which increases dopamine availability (see Fig. 10). Two laboratories (319, 320) independently generated D_2R mutants and described the consequences of D_2R loss on the neuroendocrine axis. The major phenotype is chronic hyperprolactinemia and lactotroph hyperplasia that develops into adenoma in aged females only. Pituitary PRL expression is robust in D_2R-null mice with a slight decrease in GH and no major change in other hormones. Null mice of either sex have a 3- to 4-fold higher basal PRL levels and, as expected, do not respond to the dopamine receptor antagonist haloperidol. Although D_2R mutant males have normal spermatogenesis, young females have irregular estrous cycles and lower serum estrogen levels, but with little impact on fertility. This differs from the impact of hyperprolactinemia on humans, which results in menstrual disturbances, anovulation, and infertil-

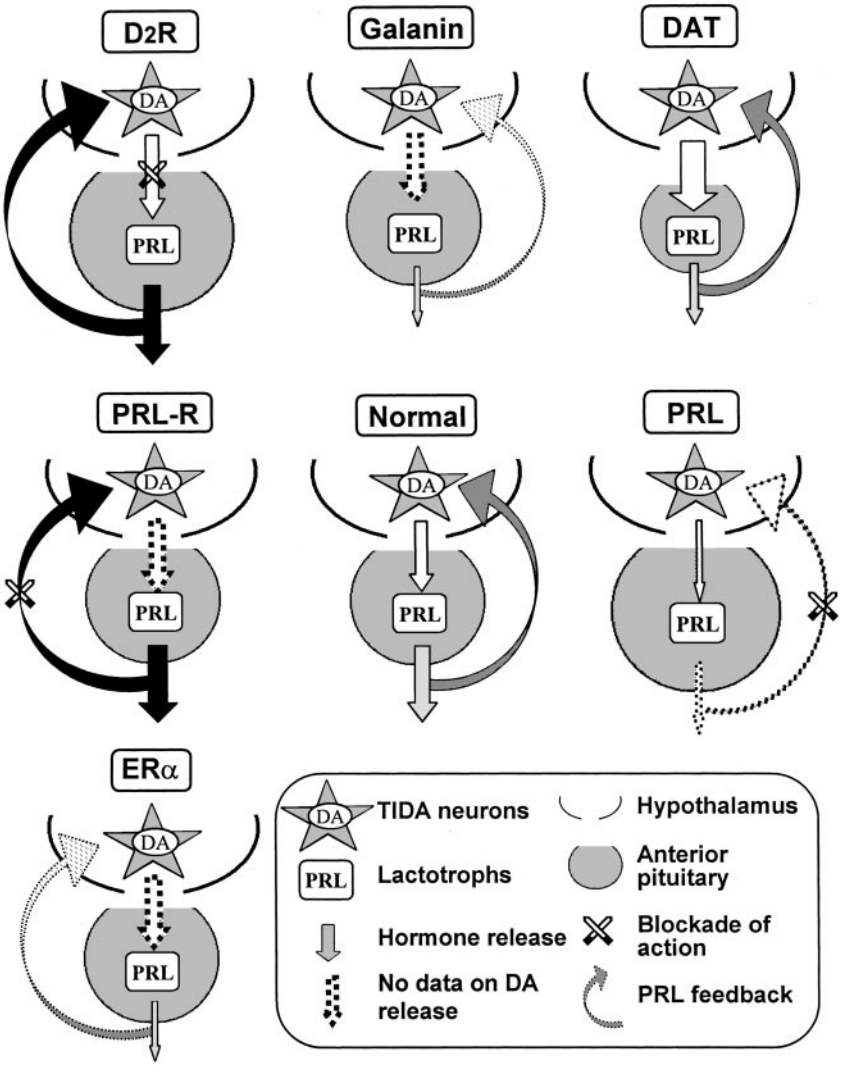

FIG. 10. Summary of the effects of various gene inactivation in transgenic mice on PRL and dopamine release and on pituitary size. The *size and color of the arrows* and the *size of the pituitary* represent increases/decreases relative to wild-type animals (normal). PRL release is increased in D_2R- and PRL-R-null mice, decreased in galanin- and ERα-null mice, and is undetectable in PRL-null mice. Pituitary size increases in D_2R- and PRL-null mice and decreases in DAT-null mice. See *Section V* for more details.

ity (321). D_2R-null females have higher serum PRL levels than males, underscoring the importance of estrogen in PRL biosynthesis. The lack of D_2R does not affect hypothalamic regulatory factors such as TRH and VIP, although this issue has not been examined in depth. Notably, pituitary PRL-R expression is also higher in the D_2R-deficient mice, perhaps secondary to pituitary hyperplasia.

Lactotroph hyperplasia, followed by adenoma formation, differs in onset and magnitude between the sexes. At 3 months of age, there is no discernible difference in pituitary size or appearance between null and normal mice. By 9–12 months, females develop extensive lactotroph hyperplasia that is barely seen in males. At this time, many dilated blood-filled spaces are seen, but there are no signs of neoplastic transformation. Older D_2R-null females (17–20 months old) develop large lactotroph adenomas, with some pituitaries increasing in size as much as 50-fold above controls (322). Whereas there is some invasion into the brain in the adenoma-bearing females, there is no metastasis. The pituitary in age-matched males only doubles in size with microscopic foci of lactotroph adenomas.

The mechanisms leading to tumor formation in females and its late onset are unknown. Although PRL itself has been implicated, there is only limited evidence that PRL is mitogenic in lactotrophs (323). On the other hand, involvement of local growth factors such as galanin, normally under dopaminergic inhibition (324), should be considered. Also, a disrupted balance between angiogenic/antiangiogenic factors such as vascular endothelial growth factor (VEGF) may be responsible for the observed perturbation in the local vasculature (325).

As expected, impairment of dopamine transmission in the nigrostriatal system results in deficits in locomotion, whereas linear growth, onset of puberty, and fecundity are not altered. Expression of D_3R, D_4R, and TH in the striatum of null mice was unaffected, whereas enkephalin mRNA levels increased. The observed effects of D_2R deletion on the intermediate lobe differ between the two laboratories, given the different genetic background of the transgenic mice. Kelly *et al.* (320) found no difference between D_2R-deficient and wild-type mice in either cell density within the intermediate lobe or β-endorphin content, whereas Saiardi *et al.* (319) reported enlargement of the intermediate lobe and increased POMC expression in null mice.

Deletion of DAT results in an almost opposite phenotype to that of D_2R deficiency (see Fig. 10). DAT-null mice have increased dopaminergic tone, anterior pituitary hypoplasia, dwarfism, and inability to lactate (112). Pituitary hypoplasia is due to decreased number of lactotrophs and somatotrophs without a change in thyrotrophs or gonadotrophs. With more dopamine presumably reaching the pituitary, one would expect a reduction in pituitary PRL content, due to down-regulation of the PRL gene, and a suppression of serum PRL levels. However, the DAT-deficient mice present two unexpected outcomes, an unchanged basal serum PRL level despite a 70–80% reduction in pituitary PRL content, and unresponsiveness to the dopamine receptor antagonist sulpiride.

Perhaps the major effect of a higher dopamine input at an early age due to DAT deficiency is on lactotroph development, selection or survival. Rapid PRL turnover in the remaining lactotrophs or increased sensitivity to PRL secretagogues may account for the maintenance of near normal basal serum PRL levels. Compensatory mechanisms acting to diminish the effects of enhanced dopamine input could be responsible for lactotroph unresponsiveness to the blockade of D_2R. These include down-regulation of the D_2R or suppression of TH, thereby reducing dopamine input to the lactotrophs. The inability to lactate despite normal circulating PRL levels could be due to several factors, including pituitary insensitivity to PRL secretagogues or insufficient pituitary PRL reserves needed for development/maturation of the mammary gland and milk production.

The mechanism leading to dwarfism in DAT-deficient mice is also of interest. There is a marked decrease in the number of somatotrophs and a reduced pituitary GH content, but circulating GH levels are normal. The expression of Pit-1 at ED 16.5 is similar in DAT-null and controls, but is 50% lower in adult DAT-deficient mice. The lower Pit-1 expression in adults may be the result of reduced number of pituitary cells expressing Pit-1 or due to suppression of Pit-1 expression by the enhanced dopamine input. Indeed, there is evidence from cultured cells that dopamine suppresses Pit-1 expression (326). Increased dopamine availability also results in lower GHRH expression with no change in somatostatin. As most GHRH neurons are located in the arcuate nucleus adjacent to the TIDA neurons (327), suppression of GHRH by elevated dopamine may decrease the supply of GHRH to the anterior pituitary, resulting in failure of somatotroph proliferation or maintenance and the resultant dwarfism.

B. PRL and PRL receptor (PRL-R)

Two laboratories have generated transgenic mice with PRL deficiencies: one resulting from deletion of the PRL-R (328) and another resulting from targeted mutation of the PRL gene (329). Unlike humans who have a single transcript, mice have 1 long and 3 short PRL-R isoforms (330). Deletion of exon 5 in the mouse PRL-R gene results in undetectable levels of the receptor protein and lack of specific binding of PRL to liver microsomes, confirming the absence of functional PRL-Rs. In contrast, the PRL gene was mutated by inserting a neomycin resistance gene downstream of the bases encoding Ser 117 in exon 4, resulting in the production of an 11-kDa immunoreactive PRL fragment by the pituitary. However, this truncated PRL is biologically inactive, as judged by the Nb2 proliferation bioassay, but can be used as a cellular marker to identify the lactotrophs. Although the truncated product is close in size to 16-kDa PRL reported to possess antiangiogenic properties (331), there is no evidence that they share similar properties.

Among the observed characteristics of these transgenic mice, we will focus on those most relevant to the neuroendocrine axis (see Fig. 10). Shared features by both mutants include irregular estrous cyclicity, absence of pseudopregnancy, abnormal mammary development, and sterility. Despite repeated mating and the presence of oviductal ova, there is an almost complete arrest of preimplantation embryo development in PRL-R-null mice. Because their fertilized

eggs develop into normal offspring in foster intact dams, a deficiency in oviductal environment is indicated rather than an abnormal oocyte development. The failure of the uterus to support blastocyst implantation, secondary to lack of PRL-supported progesterone production by the corpus luteum, renders these animals unable to maintain pregnancy. Progesterone replacement in PRL-R-null mice rescues embryo implantation and facilitates maintenance of pregnancy to term (332). Notably, the luteotropic function of PRL is unique to rodents and does not apply to most other species. Males with a disrupted PRL gene are fully fertile, whereas some males with a disrupted receptor show delayed fertility that is corrected over time. As expected, mammary gland development is abnormal in both types of mice, with unchanged ductal branching but absence of terminal or lateral lobulation.

The ligand- and receptor-deficient mice differ in several respects. One is mammary gland development in heterozygotes. Whereas mice heterozygous for PRL produce a normal litter size and have no problems nursing their young, the PRL-R-heterozygotes have impaired lactation during their first pregnancy, which is restored with successive pregnancies. This suggests that two functional alleles of the PRL-R are required for efficient mammary gland development, with hormones other than PRL (gonadal steroids, GH, and placental lactogens) providing sufficient delayed stimulation for the development of lactation-competent mammary glands. In contrast, sufficient levels of PRL can be synthesized from a single allele in PRL heterozygotes. Maternal behavior, as judged by the latency of pup retrieval and crouching, is severely impaired in the PRL-R heterozygotes (333), but appears to be normal in either homozygote or heterozygote PRL-deficient mice (329). This difference may involve PRL-like hormones (*e.g.*, placental lactogens) that act via the brain PRL-R to modify maternal behavior.

The hypothalamo-pituitary axis has not been extensively studied in either type of PRL-deficient mice. Plasma PRL in the receptor-null mice is increased 30-fold in males and 100-fold in females, with a somewhat larger pituitary size and an increased blood supply. Treatment of PRL-R-deficient mice with bromocriptine normalizes PRL levels, indicating retention of D_2R responsiveness by the lactotrophs. Although hypothalamic dopamine has not been examined in these animals, one would predict that the absence of PRL-R on the TIDA/THDA neurons results in reduced synthesis/release of dopamine as a compensatory response for a perceived lack of PRL. In the PRL-deficient females, the intensity of dopamine fluorescence and TH immunoreactivity are reduced in the A12 neurons, but the number of TIDA neurons is unchanged (334). In the PRL-deficient males, dopamine content in the median eminence, but not in the medial basal hypothalamus, is reduced by 50%, with no change in norepinephrine (335).

Perhaps the most striking, and unexpected, outcome of PRL deficiency is pituitary hyperplasia that is seen already in young (6–8 wk) males and females, developing into very large and hemorrhagic adenomas in aged females. This is similar to the late onset adenoma that develops in D_2R-knockout female mice, which have impaired dopaminergic inhibition but elevated PRL levels (Fig. 10). The common feature to the two types of transgenic mice is loss of negative growth control by dopamine, which might involve mediation by local factors such as galanin.

Transgenic mice overexpressing PRL or other lactogenic hormones have also been generated (for review, see Ref. 336). Although PRL hypersecretion for short periods can be achieved by antidopaminergic drugs or pituitary transplantation, transgenic animals overexpressing PRL offer the opportunity for exploring the physiological consequences of a lifelong exposure to excess PRL. Overexpression of the rat PRL gene in mice resulted in circulating levels of PRL as high as 250 ng/ml, a dramatic enlargement of the prostate gland (337), an increase in the expression of PRL-R in both adipose tissue and liver (338), and the development of mammary carcinomas at 11–15 months of age (338a). Transgenic mice overexpressing human GH (hGH) are also of interest because hGH has both somatogenic and lactogenic activities in rodents, resulting in physiological hyperprolactinemia and hypersomatotropism (336). Transgenic females expressing moderate amounts of hGH have low circulating PRL levels, luteal deficiency, and sterility. This appears to be due to stimulation of hypothalamic dopamine by binding of hGH to the PRL-R, resulting in a 35–50% increase in the number of TH-immunoreactive neurons in the A12, but not A13, neuronal groups (339). This effect was not seen in animals overexpressing bovine GH, which is not lactogenic.

C. ERs, galanin, and nerve growth factor (NGF)

The recent generation of ERα- and ERβ-deficient mice, named αERKO and βERKO respectively, helped in defining the diverse actions of estrogens. Most attention has been focused on the reproductive tract with only limited examination of the neuroendocrine axis that regulates PRL. Unlike the rat and human, the ERβ transcript is very low or undetectable in the normal mouse pituitary, whereas both receptors are expressed in the hypothalamus, with a predominance of ERα (for review, see Ref. 340). The αERKO males and females exhibit a 10- and 20-fold reduction in pituitary PRL mRNA levels compared with wild-type animals (see Fig. 10). The change in circulating PRL levels in females is less dramatic, showing only a 5-fold decrease, suggestive of compensatory mechanisms. The reduction in pituitary PRL expression in αERKO females is more severe than that seen in OVEX wild type mice, raising the possibility of a decreased number of lactotrophs (341). Neither TIDA/THDA neurons nor pituitary D_2R expression have been evaluated in these animals.

βERKO females have reduced fertility due to follicular arrest but exhibit normal pregnancy and lactation (342, 343). This, and the fact that ERβ is not expressed in the mouse pituitary suggest that, unlike the rat, ERβ does not play an important role in pituitary physiology in the mouse. However, because ERβ is expressed in the arcuate nucleus (194), some subtle changes in dopaminergic neurons may be evident in βERKO mice.

Galanin is a 29-amino acid peptide that is highly expressed in both the hypothalamus and pituitary gland. It is synthesized primarily by the lactotrophs where it is colocalized with PRL-containing secretory granules (344). Like PRL, ga-

lanin is very sensitive to estrogens. In estrogen-treated F344 rats, galanin expression increases 1000-fold above that in OVEX animals (344). In addition, TRH and dopamine stimulate and inhibit, respectively, galanin release from cultured anterior pituitary cells (324). Galanin-containing neurons are also found in the arcuate nucleus, and like the TIDA/THDA neurons, they project to both the median eminence and posterior pituitary (345). Galanin is involved in a number of functions, including feeding behavior, memory and cognition, and regulation of pituitary function. Galanin exerts its actions by binding to one of several receptor subtypes, all of which are members of the large family of G protein-coupled receptors, but with little homology to other neuropeptide receptors.

Recently, two types of transgenic mice were generated, one with galanin deficiency and another with pituitary-selective galanin overexpression. These mice show opposite effects on PRL production and lactotroph proliferation. The galanin-deficient mice were produced by deleting the entire coding region for galanin, resulting in undetectable levels of galanin (346). These mice are viable, grow normally, undergo puberty at the appropriate time, and are fully fertile. Pituitary PRL mRNA levels and contents in the galanin-deficient mice are reduced 30–40%, but basal serum PRL levels are unchanged (see Fig. 10). Mutant females, however, are unable to lactate, at least after the first two pregnancies, a likely result of markedly reduced plasma PRL levels. Their response to estrogen is also impaired. Similar to the situation in PRL-R-heterozygotes, subsequent pregnancies restore some of the lactation capability, suggesting delayed maturation of the mammary glands in the galanin-deficient mice. Pituitary expression of other hormones, including GH and LH, are unchanged in the mutants, as are hypothalamic VIP and TRH levels. There is no information on the status of hypothalamic dopamine in these animals.

Transgenic mice with pituitary overexpression of galanin carry a transgene composed of the mouse galanin gene ligated downstream of a 2.5-kb fragment of the rat PRL promoter (347). Both male and female transgenics have 100- to 1000-fold increases in galanin mRNA and peptide levels in the anterior pituitary, with no effects on the posterior pituitary or hypothalamus. The effect of galanin overexpression on hypothalamic hormones has not been examined. In 2- to 4-month-old transgenic females, pituitary PRL content is increased but plasma PRL levels do not rise until 6 months of age; other pituitary hormones, including GH, TSH, and LH, show little or no change. By 12 months, female pituitaries are hyperplastic with a higher number of lactotrophs and increased PRL gene expression, whereas males show no change. Estrogen is likely the driving force for these alterations, as judged by its ability to increase plasma PRL levels in the transgenic males and the suppression of PRL release after OVEX of transgenic females. About 20% of the hyperplastic pituitaries in 12-month-old females develop into adenomas. Again, the mechanism for increased lactotroph proliferation in the presence of estrogen is unclear and may involve a reduction in dopamine input, a direct action of galanin, or effects of any other local mitogenic factor that is regulated by estrogen, dopamine, or both.

NGF is a neurotropic factor known for its ability to induce and sustain a neuronal phenotype, particularly in peripheral sympathetic and sensory neurons. NGF acts via two transmembrane receptors, the high affinity trkA with an intrinsic tyrosine kinase activity, and the low affinity $p75^{NGFR}$, which functions as an enhancer of Trk receptor activity and also binds other neurotropins (for review, see Refs. 348 and 349). NGF and its receptors have been detected within the pituitary, and NGF is now considered as one of many autocrine/paracrine factors that affect pituitary function in various ways (for review, see Refs. 350 and 351). NGF is expressed in both the anterior and intermediate lobes (352), although its precise expression by the different pituitary cell types is controversial. There is immunohistochemical evidence for colocalization of NGF in storage vesicles within the lactotrophs, supported by the fact that VIP and the dopamine agonist quinpirole stimulated and inhibited, respectively, the secretion of both PRL and NGF (353).

Transgenic mice overexpressing NGF in the anterior pituitary were generated by fusing the mouse NGF cDNA to the PRL promoter (354). These transgenics were created to examine whether ectopic NGF would attract innervation to the anterior pituitary. Although the overexpressed NGF failed to induce pituitary innervation, it caused dramatic lactotroph hyperplasia, with the pituitary enlarged more than 50-fold above its normal size. The effect of NGF overexpression on dopamine neurons and D_2R expression have not been determined in these studies. Later studies provided evidence that NGF plays a role in mitosis and differentiation of the lactotrophs, especially during early neonatal life (355, 356).

VI. Clinical Aspects

A. PRL physiology in humans

The physiology of PRL in humans differs considerably from that in rodents and will be briefly summarized (see also Fig. 11). During embryogenesis, lactotrophs are derived from progenitor bihormonal somatomammotrophs and are the last pituitary cell type to differentiate. Although GH-producing cells are detectable in the fetal pituitary at 10 wk gestation, an appreciable number of PRL-positive cells is seen only at 15–17 wk (for review, see Refs. 357–360). Pituitary PRL content increases steadily from mid to late gestation without an apparent difference between the sexes. Lactotrophs comprise 10% of the total pituitary cell population at 15 wk, increase to 15% and 30% at 20 and 36 wk, respectively, and reach up to 40% of total pituitary cell population at delivery (357). Despite the scarcity of PRL-positive cells in the early embryo, PRL is detectable as early as 5–6 wk gestation in fetal pituitary cultures and in umbilical cord plasma. Fetal plasma PRL levels are low between 10 and 20 wk gestation and rise progressively to peak levels before birth. An additional rise occurs immediately after birth, persisting for several days and declining during the first postnatal month. During childhood, there are no sex differences in serum PRL concentrations, but concentrations are twice as high in women relative to men after puberty.

It is unclear whether input from the fetal hypothalamus affects lactotroph differentiation and PRL synthesis in the

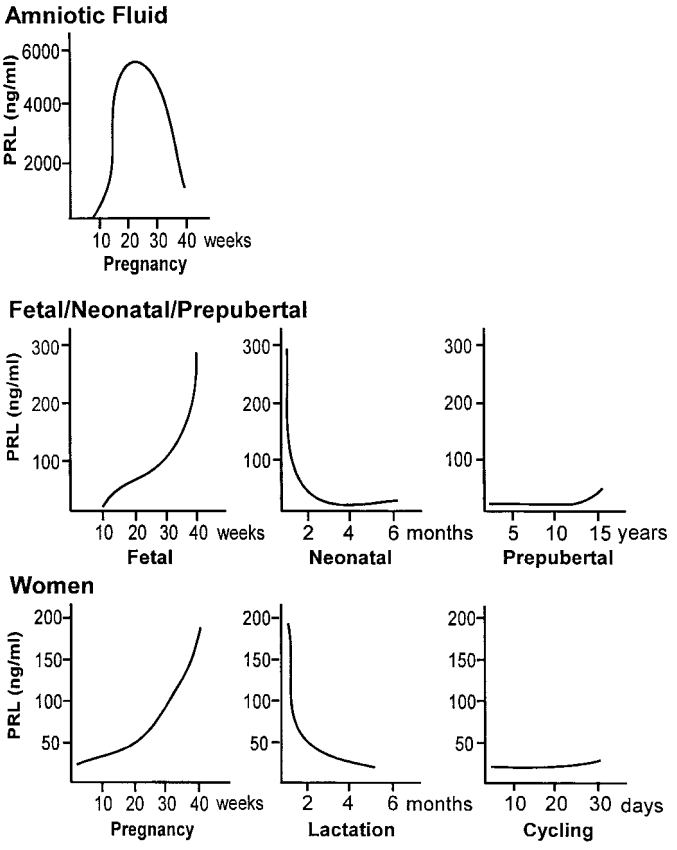

FIG. 11. Schematic presentation of PRL levels in humans under various physiological conditions. The levels shown are approximations and do not reflect acute changes such as diurnal variations or suckling-induced rises. Note the different scale for amniotic fluid PRL.

early human embryo. Catecholamine-positive neurons are seen in the arcuate nucleus and the external layer of the median eminence at 12–16 wk gestation, and human fetal hypothalami taken during this time suppress PRL release when cultured with rat pituitaries (361). However, it is unknown whether fetal lactotrophs express dopamine receptors or respond to dopamine at this time. PRL in cord blood from newborns whose mothers were treated with bromocriptine is significantly suppressed (362). Additionally, infants undergoing cardiovascular surgery respond to dopamine infusion with an immediate drop in serum PRL levels, followed by a rebound the next day (363). Thus, at least during the perinatal period, and possibly earlier, human lactotrophs respond to dopaminergic inhibition. In general, PRL release in the fetus appears to be independently regulated, based on the poor correlation between PRL levels in the maternal and fetal circulations, the limited transport of PRL between the two compartments, and the differential response of maternal and fetal PRL to dopamine drugs (364).

PRL in the maternal circulation increases progressively toward term, when its concentration is 7- to 10-fold higher than that in nonpregnant women (Fig. 11). These high PRL levels are essential for the preparation of the breast for the initiation of lactation, which is prevented during pregnancy by the high levels of progesterone. Pregnancy is also associated with lactotroph hypertrophy and hyperplasia, accounting for a considerable enlargement of the pituitary. There is also a marked increase in pituitary vascularization, which may be the consequence of increased VEGF production by lactotrophs (325, 365). The role of estrogens in these processes is presumed but unproven. Active labor initiates a bimodal change in maternal serum PRL levels, an initial drop followed by a rise immediately after delivery that lasts for several hours (366). Neural signals from the cervix trigger the PRL surge during labor, because it is not seen during cesarean delivery (367), but its functional significance is unknown.

During pregnancy, large amounts of PRL are independently produced by the decidua (for review, see Ref. 368). Although decidual PRL is identical with pituitary PRL (except for a higher ratio of glycosylated PRL), its expression is driven by an alternative promoter (369) and its release is regulated by an entirely different control mechanism. Unlike the pituitary lactotrophs, which possess dense secretory granules, decidual cells have little storage capacity. Indeed, decidual PRL is rapidly released after synthesis and is not affected by increased extracellular calcium. This is unusual for cells that secrete protein hormones and is more typical of steroid-producing cells. Neither dopamine nor estrogen affect PRL release from decidual explants (370). Progesterone is mandatory for decidualization but does not act as a PRL secretagogue *per se* (371). IGF-I, insulin, and relaxin stimulate decidual PRL release, whereas lipocortin-1 and endothelins and several interleukins are inhibitory (for review, see Ref. 368). Decidual PRL is secreted into the amniotic fluid (see Fig. 11), where its concentrations peak between 18–23 wk gestation, attaining 50- to 100-fold higher levels than those in either the fetal or maternal circulation (372, 373). Despite the intimate contacts between amniotic fluid PRL and the developing fetus, its role in fetal physiology is unclear.

Basal PRL levels remain elevated during the first 2–3 wk postpartum in women who do not breast feed and then decline (Fig. 11). Suckling is the most potent and best characterized physiological stimulus for PRL release. The magnitude of the suckling-induced PRL rise is robust during early lactation but wanes thereafter (374). Tactile stimuli of the breast can increase serum PRL in nonlactating women but not in men (375). Lactational amenorrhea, used by some postpartum women as a method of contraception, appears to be more associated with the frequency and duration of the suckling episodes rather than due to a persistent hyperprolactinemia (376).

Estrogens are responsible for the higher basal serum PRL levels, the enhanced responsiveness to PRL secretagogues, and the higher incidence of prolactinomas in women than men. In contrast to rats, estrogens have little effect on acute PRL release in humans. Circulating PRL levels do not increase during the preovulatory LH surge and are basically unchanged throughout the menstrual cycle (377). Also, oral contraceptives do not appreciably increase serum PRL levels and do not contribute to prolactinoma development or growth. There are only a few studies on the *in vitro* effects of estrogens on human lactotrophs. Cultured lactotrophs from midterm fetal pituitaries do not respond to E2 (358), although they already express both ERα and ERβ at this time (378). However, E2 increases PRL release from acutely dispersed adult human pituitary cells, but does not reverse the

dopamine-mediated inhibition (378a). This indicates that estrogen is a more effective stimulator of lactotrophs when the dopaminergic inhibition is absent or reduced. Studies with monkeys also suggest that there is no simple causal relationship between estrogen and PRL release (360).

Stress conditions, including anesthesia, surgery, electric shock, strenuous exercise, and insulin-induced hypoglycemia, also stimulate PRL release in both men and women (for reviews, see Refs. 360 and 379). There are also small increases in PRL in response to high protein meals, attributed to the specific action of phenylalanine and tyrosine (380). Administration of naloxone, phentolamine, or propranolol does not alter the PRL response to test meals, indicating that neither opioid, α-adrenergic, nor β-adrenergic stimulation mediate the meal-induced PRL release. The diurnal changes in circulating PRL levels in humans are also of interest. When sampled at high frequency, serum PRL levels exhibit an episodic pattern of release with as many as 5–15 secretory episodes per day. The amplitude of these pulses increases within 60–90 min after the onset of sleep, occurring primarily during the non-rapid eye movement periods (381). Unlike the situation in the rat, the diurnal variation in PRL secretion is evident in both men and women and is sleep induced rather than driven by an inherent diurnal rhythm.

B. Drug-induced PRL release

Drugs that interfere with dopaminergic neurotransmission, either by increasing its availability or by reducing its effectiveness, often, but not always, affect PRL homeostasis. The following diseases, which require long-term treatment with dopamine-altering drugs, will be briefly discussed: Parkinson's, schizophrenia, depression, and hypertension (see summary in Table 1). In addition, opioidergic drugs that interact with the hypothalamic dopaminergic system are used for alleviating chronic pain and for pain management in cancer patients. The effects of these medications on PRL release vary with the type of drug, doses, and duration of treatment (for review, see Ref. 382).

Parkinson's disease is a neurodegenerative disorder of the dopaminergic nigrostriatal pathway, resulting in dopamine depletion in the basal ganglia and a relative excess of cholinergic activity. The main manifestation of Parkinson's disease is movement disorders consisting of tremor, rigidity, and akinesia, with depression and dementia occurring in some older patients. Although the pathogenesis of primary Parkinson's disease is unclear, genetic, viral, and environmental toxins have been implicated (reviewed in Ref. 383). The latter has been reinforced by the discovery that an illegal "designer drug," 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), produces irreversible and selective destruction of the substantia nigra dopaminergic pathway, resulting in a Parkinsonian syndrome (reviewed in Ref. 384).

Perhaps due to the lack of dopamine autoreceptors, a sluggish reuptake mechanism, or protective effects of local neurotropic factors, the function of the TIDA neurons is conserved in Parkinson's disease and untreated patients have normal basal, as well as episodic, PRL release (385). L-Dopa, which can cross the blood brain barrier, remains the mainstay of therapy for replenishing dopamine, but unfavorable side effects such as fluctuations in motor performance and psychosis have led to the development of new dopaminergic and nondopaminergic drugs. Among the former are D_2R agonists such as bromocriptine, lisuride,

TABLE 1. Selected drugs used in clinical practice that affect PRL secretion

Disease/Drug	Effects on PRL	Category	Mechanism of Action
Hyperprolactinemia			
Bromocriptine	Strong suppressor/short duration	Dopamine agonist	D_2R activator
Pergolide	Strong suppressor/medium duration	Dopamine agonist	D_2R activator
Cabergoline	Strong suppressor/long duration	Dopamine agonist	D_2R activator
Schizophrenia			
Haloperidol	Moderate/chronic stimulator	Neuroleptics	D_2R blocker
Perphenazine	Moderate stimulator	Neuroleptics	D_2R blocker
Risperidone	Strong stimulator	Atypical neuroleptics	5-HT2 and D_2R antagonist
Parkinson's disease			
L-Dopa	Suppressor/short duration	Dopamine precursor	Increases dopamine
Pergolide	Suppressor/medium duration	Dopamine agonist	D_2R activator
Cabergoline	Suppressor/long duration	Dopamine agonist	D_2R activator
Depression			
Moclobemide	Short-term stimulator	MAO-A inhibitor	Increases serotonin
Desimipramine	Short-term stimulator	Antidepressant	Serotonin reuptake inhibitor
Paroxetine	Delayed stimulator	Antidepressant	Serotonin reuptake inhibitor
Chronic pain			
Morphine	Short-term stimulator	Analgesic	μ-Opioid receptor agonist
Spiradoline	Moderate stimulator	Antinociceptive	κ-Opioid receptor agonist
Cardiovascular disease			
Verapamil	Chronic stimulator	Calcium channel blocker	Suppresses dopamine
Methyldopa	Short-term stimulator	Antihypertensive	DDC inhibitor
Reserpine	Moderate chronic stimulator	Antihypertensive	Dopamine depletion
Gastric disorders			
Metoclopramide	Strong acute stimulator	Antiemetics/challenging tests	D_2R antagonist
Domperidone	Moderate stimulator	Prokinetics	Peripheral D_2R antagonist
Miscellaneous			
Fenfluramine	Strong acute stimulator	Challenging tests	Serotonin release/reuptake inhibitor
Methadone	Moderate chronic stimulator	Antiaddictive	μ-Opioid receptor agonist

pergolide, and cabergoline (see Table 1). Several studies have assessed the effects of long term L-DOPA therapy alone or in combination with D_2R agonists on the neuroendocrine system (92, 386). They found suppressed serum PRL and TSH levels and elevated GH levels but unchanged PRL responsiveness to TRH. Because there are no documented effects of prolonged hypoprolactinemia in humans, it can be concluded that treatment of Parkinson's patients with dopaminergic agonists has minimal prolonged consequences on the physiology of PRL.

Schizophrenia is a life-long mental illness with an early onset in teens and young adults that affects as much as 1% of the world's population. Its etiology is complex, involving genetic predisposition, perinatal developmental factors, viral infection, and nutritional deficiency (for review, see Refs. 387 and 388). Clinical manifestations of schizophrenia are rather broad, including hallucinations and delusions, disorders of thought as well as of cognitive and social behavior, and some physical abnormalities. Males are more frequently and more severely affected than females. Unlike Alzheimer's or Parkinson's disease, both of which are characterized by well-defined neurotransmitter deficiency or recognizable neuropathological markers, the anatomical/neurochemical abnormalities in schizophrenia are obscure and widespread. Reported structural or functional abnormalities in schizophrenic patients include enlargement of the ventricles, decreased cerebral blood flow, and morphological alterations in the hippocampus and prefrontal cortex. Nonetheless, the general consensus is that schizophrenia is a functional rather than a neurodegenerative disorder.

Hyperdopaminergia is a prominent theory proposed over 40 yr ago in an attempt to explain the neurochemical basis of schizophrenia (for review, see Refs. 76, 389, and 390). This theory was based on the effectiveness of antipsychotic drugs that block dopamine receptors to alleviate many symptoms of the disease, whereas drugs that increase dopamine availability, *e.g.*, L-DOPA, and dopamine reuptake inhibitors such as cocaine and amphetamine, produce schizophrenic-like psychoses. There is also some evidence from postmortem specimens for higher concentrations of dopamine and homovanillic acid and increased density of D_2R in certain brain regions of schizophrenics, findings that are supported by CSF sampling (391) and *in vivo* neuroimaging (392) in subsets of patients. More recent theories, while not disputing the central role played by dopamine, extend the putative dysfunctions in schizophrenia to alterations in neural circuitry involving serotonin, glutamate, and cholinergic neurotransmitters among others (76, 390).

The first-generation neuroleptics shared an ability to suppress dopamine neurotransmission and included drugs such as chloropromazine, haloperidol, and trifluoperazine. Although classical neuroleptics were generally effective in treating delusions and hallucinations, they fell short of improving many cognitive deficits. Moreover, they invariably caused severe sedative, hypotensive, and extrapyramidal (Parkinson's-like) side effects (393). Most classical neuroleptics also induce significant increases in serum PRL levels (see Table 1), although few studies observed no changes (reviewed in Ref. 394). Some of the discrepancies between studies can be explained by differences in the responsiveness between men and women, drug specificity, the ratio of bioactive to immunoreactive PRL, and the presence of serum PRL binding proteins (395–397). Amenorrhea in some female patients and sexual dysfunction in some men are also indicative of increased circulating PRL levels in response to neuroleptics (394).

The adverse effects of neuroleptics, together with the recognition that hyperactivity of dopaminergic neurons does not fully explain schizophrenia, have led to the development of a second generation of antipsychotic drugs, the atypical neuroleptics. These drugs, which include clozapine, risperidone, and olanzepine among others, do not produce significant extrapyramidal side effects, although they still cause some undesirable sedative and hypotensive effects (393). The improved efficacy of these drugs on cognitive functions is attributed, in part, to a high serotonin-to-dopamine receptor blockade ratio. Most of these drugs produce little or no clinically significant PRL elevation except for risperidone, which is comparable to classical neuroleptics in its proclivity to raise serum PRL levels (394).

Serotonin receptor agonists and reuptake inhibitors are widely used in the treatment of depression. Studies with experimental animals established that drugs that increase serotonergic and opiodergic efficacy stimulate PRL release by suppressing the hypothalamic dopaminergic system (reviewed in Refs. 105, 113, and 253). However, with the exception of the serotonin reuptake inhibitors D-fenfluramine and desimipramine, which increase PRL release (see Table 1), most other serotonergic drugs have little effect on circulating PRL levels in humans (398). On the other hand, monoamine oxidase inhibitors such as moclobemide, also used as antidepressants, induce both acute and prolonged rises in plasma PRL levels (399).

Another class of drugs with a potential to affect PRL release are the opioid modulating drugs used in pain management (Table 1). Early studies reported that morphine, methadone, and met-enkephalin analogs increase circulating PRL levels in normal male volunteers (400). More recent studies found gender differences in the response to some of these drugs (401), and a preferential activation of the κ-opioid receptors (402). A few other drugs in clinical practice also affect PRL release. Among these are the D_2R antagonists metoclopramide and domperidone, used in the treatment of gastric motility disorders (403), verapamil, a calcium channel blocker used in the treatment of cardiovascular disease (404), and α-methyldopa and reserpine, used in the treatment of hypertension (405).

C. Hyperprolactinemia and pituitary prolactinomas

Pathological hyperprolactinemia is defined as a consistent elevation of serum PRL levels above 20 ng/ml in nonpregnant, nonlactating individuals (reviewed in Refs. 321, 406, and 407). After excluding drug effects, hypothyroidism, chronic renal failure, and cirrhosis, elevated serum PRL levels are highly predictive of hypothalamic disease or pituitary tumors. The term "hypothalamic disease" covers several disorders that include tumors (*e.g.*, craniopharyngiomas, gliomas, or meningiomas), vascular disturbances, and trauma to the pituitary stalk, all of which interfere with dopamine

delivery to the pituitary. With respect to pituitary tumors, a distinction should be made between prolactinomas that release excess amounts of PRL and large adenomas that may compress the pituitary stalk or invade into the hypothalamus, resulting in increased PRL release due to dopamine deprivation. Radiologically diagnosed large adenomas with serum PRL levels below 100–150 ng/ml are suggestive of non-PRL secreting tumors rather than prolactinomas. On the other hand, the levels of PRL that are secreted by prolactinomas are usually proportional to tumor size and can reach circulating levels as high as 5,000–10,000 ng/ml.

Pituitary tumors are nonmetastasizing adenomas with diverse hormonal, cytological, and proliferative characteristics. In addition to primary classification according to the secreted hormone, they are subclassified by size, anatomical location, and invasive properties. The incidence of adenomas in the general population, based on radiological and postmortem analysis, has been estimated as high as 15–20%, but most are asymptomatic (for review, see Refs. 407 and 408). Clinically diagnosed pituitary adenomas represent 10% of all intracranial tumors. Prolactinomas or mixed PRL-/GH-secreting tumors are the most common pituitary adenomas, with an overall higher incidence in women then men. Prolactinomas are generally divided into three categories: microprolactinomas (<10 mm in diameter), macroprolactinomas (>10 mm in diameter), and macroprolactinomas with extrasellar invasion. Although invasive prolactinomas can compress the optic chiasm, cause hypothalamic damage, and erode the third ventricle, they are seldom metastatic, and true carcinomas of pituitary origin are very rare.

Two hypotheses were put forward to explain the pathogenesis of prolactinomas: dopamine dysregulation and local somatic mutation (for review, see Refs. 408 and 409). Prolactinoma formation due to loss of dopamine inhibition is supported by several observations. Among these are development of large prolactinomas in transgenic mice with D_2R deletion (322), and estrogen-induced prolactinomas in Fischer 344 rats that involve pituitary neovascularization and an escape from dopaminergic inhibition (410). In humans, the resistance of some prolactinomas to dopamine has been taken as evidence for altered dopaminergic receptor or postreceptor regulatory control. On the other hand, prolonged dopamine deficits in humans caused by neuroleptics or pituitary stalk dysfunction do not induce prolactinomas. Also, most tumors are confined to a portion of the pituitary rather than presenting as a widespread hyperplasia. Further, there is a relatively low frequency of recurrence after successful tumor resection. Collectively, these observations argue against loss of dopamine inhibition as the primary cause of prolactinoma formation in humans despite evidence to the contrary obtained in animal studies.

The local somatic mutation hypothesis is based on X-chromosomal inactivation analysis showing that almost all human pituitary adenomas are monoclonal (411, 412). This is consistent with tumor origin from a single cell that has undergone genomic mutation(s) followed by clonal expansion. The clonality of adenomas has prompted a search for genetic abnormalities such as loss of tumor suppressor genes or activation of protooncogenes that may explain the abnormal cell proliferation. However, alterations in p53 and retinoblastoma genes are very uncommon in prolactinomas, as are rearrangement, amplification, or mutations of oncogenes such as myc, ras, c-erbB2 (neu), and G_α proteins. Interestingly, somatic mutations in G proteins have been identified in 30–40% of GH-secreting adenomas (409). Despite evidence for loss of heterozygocity on the long arm of chromosome 11, where the multiple endocrine neoplasia type 1 (MEN1) tumor suppressor gene is located, inactivation of this gene in sporadic pituitary adenomas is rare (413). Recently, a pituitary tumor transforming gene, originally cloned from GH3 cells (414), was found to be over-expressed in most PRL- and GH-secreting human pituitary adenomas (415). Further examination showed that the pituitary tumor transforming gene (PTTG) induces expression of fibroblast growth factor-2, may promote angiogenesis, and is expressed in several types of human cancer (416), but its potential role in the pathogenesis of prolactinomas remains to be determined.

In view of these conflicting findings, an integrated approach that reconciles the two theories has emerged (408). This theory stipulates genomic alteration (the identity of which remains unknown) that transforms a single lactotroph as the initial step. The transformed cell escapes normal cell cycle regulation and starts proliferating in response to local growth factors that are abundant in the pituitary such as fibroblast growth factor, EGF, TGFα, or TGFβ. Given that the transformed cells proliferate faster than normal cells, they are at an increased risk of additional genetic alterations, further promoting tumor progression. Among these could be loss of responsiveness to dopamine due to mutations in the D_2R or its signaling pathways. Although some reduction in D_2R expression and receptor density has been reported in prolactinomas (79), this applies only to the 10–15% of tumors that are resistant to bromocriptine therapy and does not offer a universal mechanism that explains the pathogenesis of prolactinomas. It is also important to emphasize that microprolactinomas in most patients grow very slowly and do not normally progress to become macroprolactinomas (407), suggesting a different etiology of the two types of tumors.

Gonadal dysfunction leading to infertility is a major clinical manifestation of hyperprolactinemia in women. Large tumors may also cause neurological symptoms such as headache and visual field disturbances that are related to tumor mass (for review, see Refs. 321, 406, and 417). Galactorrhea, or inappropriate milk production, is seen in a large percentage of hyperprolactinemic women, although it can also occur in normoprolactinemic women. Common symptoms of hyperprolactinemia in women of reproductive age are amenorrhea, oligomenorrhea, and infertility. High PRL levels are believed to inhibit release of gonadotropins (418), presumably by affecting GnRH pulsatility. This is supported by the detection of PRL-R in the GnRH-expressing GT1 neuronal cell line and the suppression of GnRH release from these cells by PRL (419). Direct effects of hyperprolactinemia on both the pituitary and ovary have also been postulated (321, 420). Other, less frequent, symptoms are decreased libido and increased anxiety and depression. Hypoestrogenism associated with hyperprolactinemia can result in osteopenia in premenopausal women, but hyperprolactinemia may decrease bone density independently of the hypoestrogenic state (421). Bone density may be re-established in many

women upon restoration of normal menstrual function with dopamine agonists.

Macroprolactinomas are more common in men than women. It is unclear, however, whether this is due to a true gender-specific difference in tumor pathogenesis or because of delayed diagnosis in men compared with women that are presented with menstrual irregularities (422). About 80–90% of men with prolactinomas experience some sexual dysfunction, such as diminished libido and/or impotence. The relatively low serum T levels and some decrease in sperm counts and motility are generally attributed to decreased pulsatile secretion of LH and FSH, although infertility is not a major clinical presentation. Different studies report that between 5–20% of males with impotence are hyperprolactinemic, but there is no ready explanation for the mechanism by which hyperprolactinemia causes erectile dysfunction (423). A small percentage of men with prolactinomas (10–20%) also have galactorrhea.

D. Treatment of hyperprolactinemia

Once hyperprolactinemia is confirmed by hormone assays, radiological evaluation by means of magnetic resonance imaging or high resolution computed tomography is essential for verifying the presence or absence of pituitary adenoma. Microprolactinomas, which normally grow very slowly, can be left untreated if the symptoms are not bothersome and the patient does not wish to correct menstrual disturbances, infertility, sexual dysfunction, or hypoestrogenism. On the other hand, macroprolactinomas often cause neurological problems in addition to functional hyperprolactinemia and should normally be treated. One rare complication of macroadenomas is pituitary apoplexy, a severe form of acute intratumoral hemorrhage that can be life threatening and may lead to permanent hypopituitarism (reviewed in Ref. 424). Apoplexy is presented as a sudden onset of headache, visual impairment, nausea, and disturbance of consciousness, often warranting immediate neurosurgery for tumor decompression. Although apoplexy can occur spontaneously, predisposing factors include obstetrics hemorrhage (Sheehan syndrome), anticoagulant therapy, cardiac surgery, brain angiography, head trauma, and hypothalamopituitary function tests with clomiphene, TRH, and GnRH (425, 426).

The treatment of prolactinomas includes surgical resection, radiation, and therapy with dopamine agonists. Several large studies reveal that transsphenoidal surgery for tumor removal can result in normal plasma PRL levels in 60–70% of patients with microprolactinomas and in 25–30% of those with macroprolactinomas (for review, see Refs. 406 and 407). The mortality rate is less than 1%, and major complications of surgery include CSF rhinorrhea and transient diabetes insipidus. The larger the tumor, the lower the curative success rate and the higher the recurrence rate. Pituitary irradiation in the treatment of prolactinomas is less common, and data of its success are more limited. Since the early 1970s, surgery with or without radiotherapy has been progressively replaced by dopamine agonist therapy and is now generally reserved for patients who do not respond to dopamine, are intolerant of dopamine agonists, or do not wish to undergo years of medical therapy.

Bromocriptine was the first dopamine agonist to be widely used in the treatment of hyperprolactinemia (see Table 1). This ergot alkaloid has been introduced into medical practice after extensive characterization of its binding to dopamine receptors, inhibition of PRL secretion *in vitro* and *in vivo*, and suppression of tumor size in animal studies (for review, see Ref. 427). Treatment of hyperprolactinemic women with bromocriptine results in normoprolactinemia and return to ovulatory menses in 70–90% of patients (428). A similar success rate in correcting serum PRL levels and sexual dysfunction has been reported for male patients treated with bromocriptine (422). The effectiveness of bromocriptine in reducing tumor size varies among patients and length of treatment and does not always correlate well with circulating PRL levels. The 5–15% of tumors that do not respond to bromocriptine appear to be due to low expression of D_2R and possibly result from a decrease in the relative proportion of the short receptor isoform (429).

Nausea, vomiting, and orthostatic hypotension are among the most common side effects of bromocriptine therapy. These are usually transient and can be alleviated by adjusting the doses; further improvement has been achieved by intravaginal administration of bromocriptine. As expected, bromocriptine administration may exacerbate preexisting schizophrenia. Potential adverse effects of bromocriptine therapy in pregnant women with prolactinomas have been of special concern. However, data collected to date show no increase in miscarriages, ectopic pregnancies, congenital malformation, or long-term effects on the offspring (407).

Several other dopamine agonists, *e.g.*, pergolide and lisuride as well as the more selective D_2R receptor agonists quinegolide and cabergoline (Table 1), have been used in the pharmacotherapy of prolactinomas (407, 417). Pergolide has been extensively used for treating Parkinson's disease but has also been proven as effective as bromocriptine in the treatment of hyperprolactinemia, with some variation among patients. In several recent studies, cabergoline, an ergoline derivative, has been rated as good or better than bromocriptine for normalizing serum PRL levels as well as for inducing tumor shrinkage (430). The main advantage of cabergoline is its long-lasting properties, having a duration of action up to 2–3 wk after a single oral dose. Because cabergoline has not been in practice for an extended period of time, it is not recommended for treatment of fertility until its safety during pregnancy and the question of potential teratogenicity are resolved (407).

VII. Summary and Perspectives

Although dopamine is a small and relatively simple molecule, it fulfills many diverse functions. Within the brain, it acts as a classical neurotransmitter that exerts its actions within seconds. Yet, its attenuation or overactivity can lead to some of the most protractive neurological and psychological disorders. Within the pituitary, dopamine suppresses the high intrinsic secretory activity of lactotrophs. As this requires a continuous input of dopamine,

the hypothalamic dopaminergic neurons differ from their striatal counterparts by being constitutively active. Acting via type 2 receptors that are functionally linked to membrane channels, dopamine rapidly inhibits PRL release from storage vesicles by controlling calcium fluxes. In addition, the G protein-linked receptor activates several interacting signaling pathways, resulting in the inhibition of PRL gene expression and the suppression of lactotroph proliferation. Although the mechanism of cell growth suppression by dopamine is not completely understood, this property has been exploited in the treatment of patients with prolactinomas and the restoration of their reproductive or neurological functions. In fact, the transfer of dopaminergic agonists/antagonists from the research bench to clinical practice and the generation of more effective and selective dopaminergic drugs is one of the most rewarding outcomes of basic research in this field.

The introduction of more refined detection and visualization techniques has been instrumental in attaining a more precise tracing of the hypothalamic dopaminergic pathways and a better delineation of their overlapping effects on pituitary function. Yet there remain several unresolved issues. For example, not all rises in PRL can be explained by reciprocal changes in dopamine production or secretion. Thus far, little is known about the relative importance and interactions between dopamine and a wide variety of compounds that stimulate PRL, generally known as PRFs. It remains a mystery whether there is a singular and predominant PRF, and if so, why 40 yr of research have failed in its isolation and identification.

Over the last decade, the field of dopamine-PRL interactions has advanced from a descriptive into a mechanistic phase. This has been aided by the cloning and characterization of the various receptors and transporters and the generation of transgenic animals with deleted or overexpressed genes that affect dopamine or PRL. However, because PRL is an adaptive hormone whose functions differ substantially among species, extrapolation of results obtained from transgenic mice should be done with caution. This is particularly true for the regulation and function of PRL in humans. For instance, knowledge of the role of PRL during human embryonic development is hampered by the lack of animal models with highly elevated PRL levels during pregnancy. Estrogen, known as a potent regulator of PRL secretion in rodents, has little acute effects on PRL in humans. Although some indirect evidence suggests that PRL is involved in immune regulation, mice with mutated PRL or PRL-R appear to have a normally functioning immune system. Whether this is true in other species, including humans, is presently unknown.

There are several areas in which future research may expand: 1) the mechanism underlying constitutive activation of the hypothalamic dopaminergic neurons and the hierarchy of their interactions with other neurotransmitters and neuropeptides; 2) the function of PRL as a neurotropic and survival factor for either the dopaminergic neurons or surrounding glia and the mechanism by which PRL is transported from the pituitary into the brain; 3) the origin and regulation of dopamine within the anterior pituitary; 4) the mechanism by which dopamine and its agonists inhibit lactotroph proliferation; and 5) the etiology of pituitary prolactinomas and the introduction of alternative treatments such as targeted gene therapy for their eradication.

Acknowledgements

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